

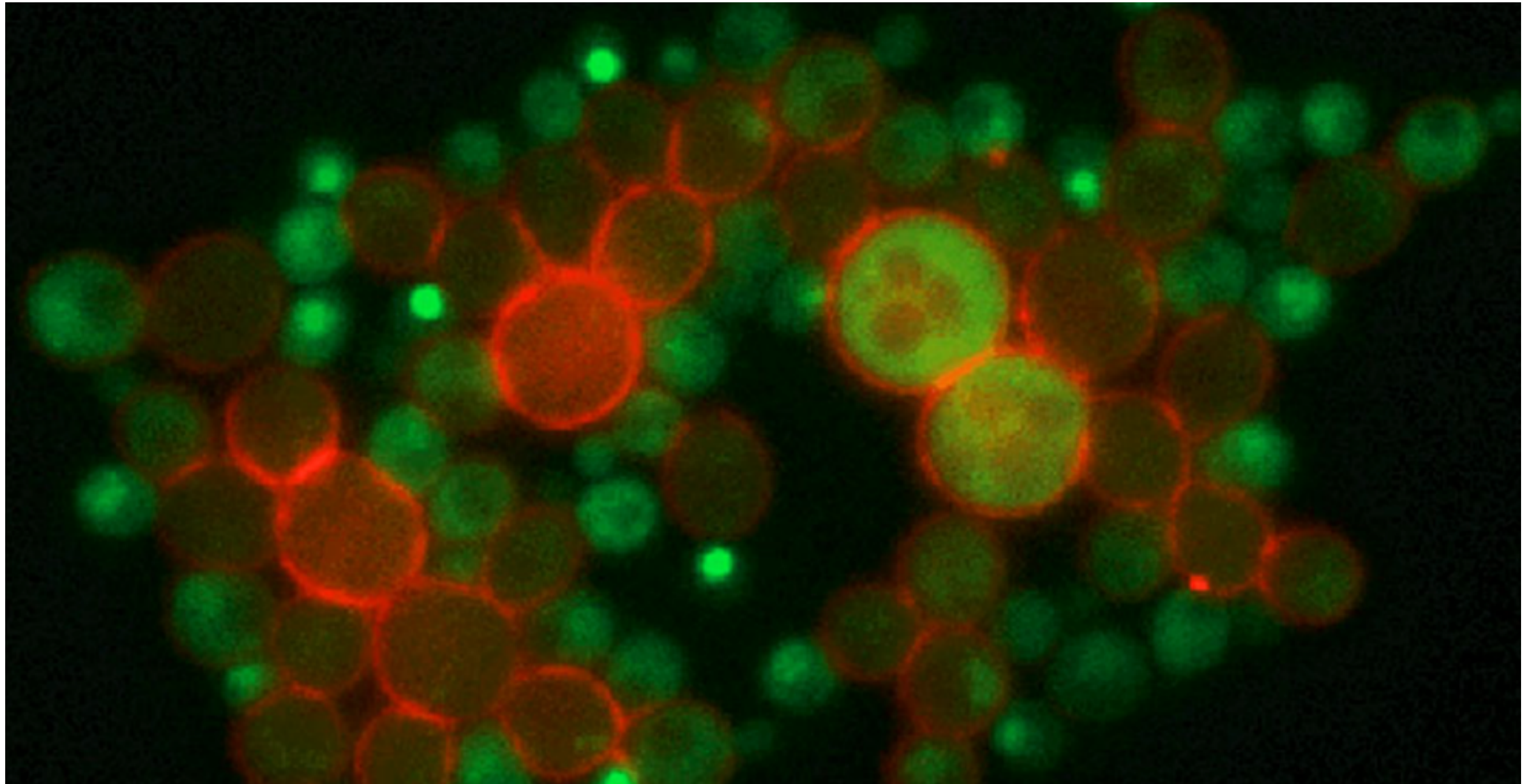
Understanding Distant Causation

How can observations at one level
reveal specifics of causation at a
more detailed level?

Randy Phillis
Department of Biology
UMass Amherst



GFP - Green Fluorescent Protein
RFP - Red Fluorescent Protein



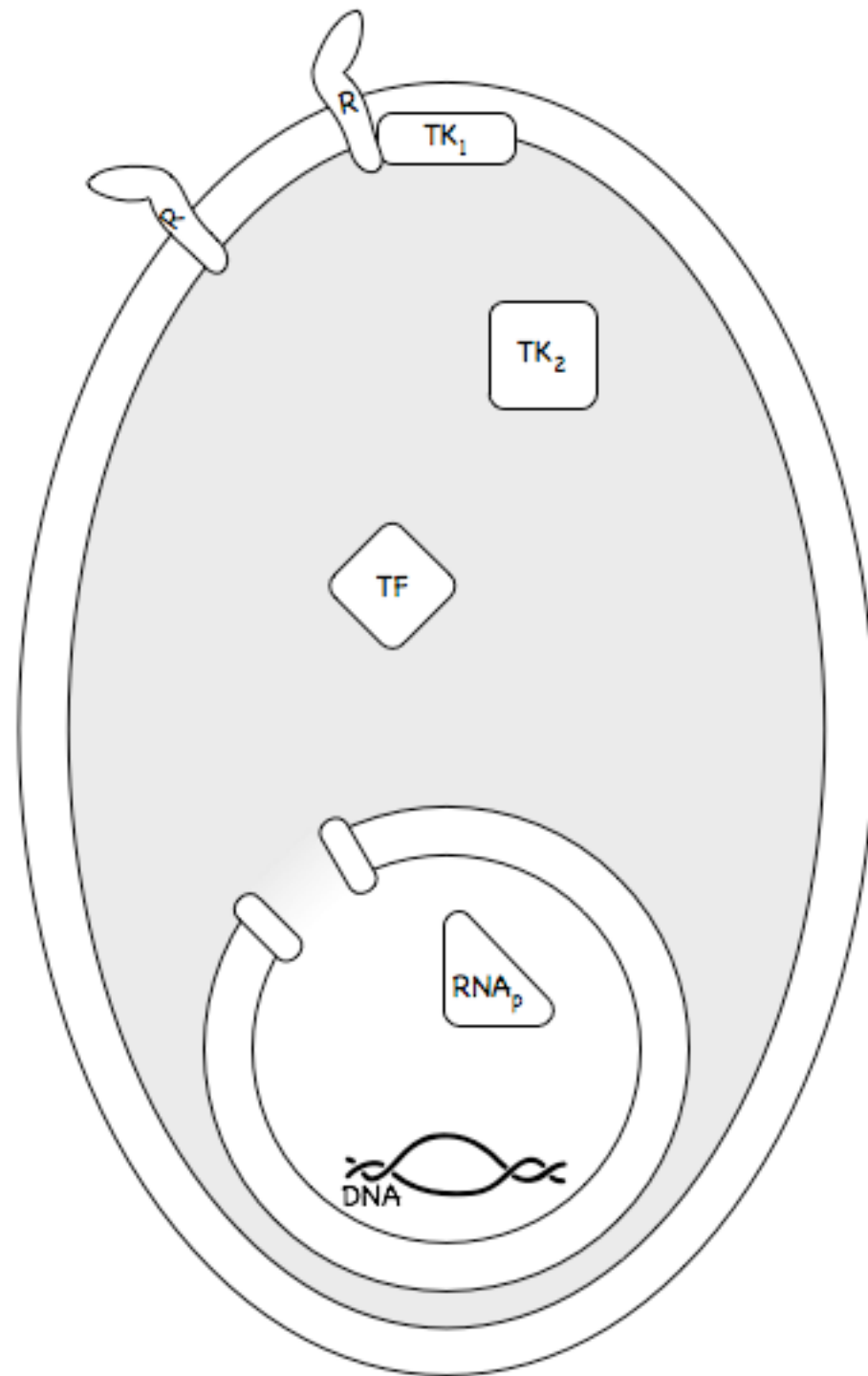
Learning Objectives

Engage complex system spanning multiple related models.

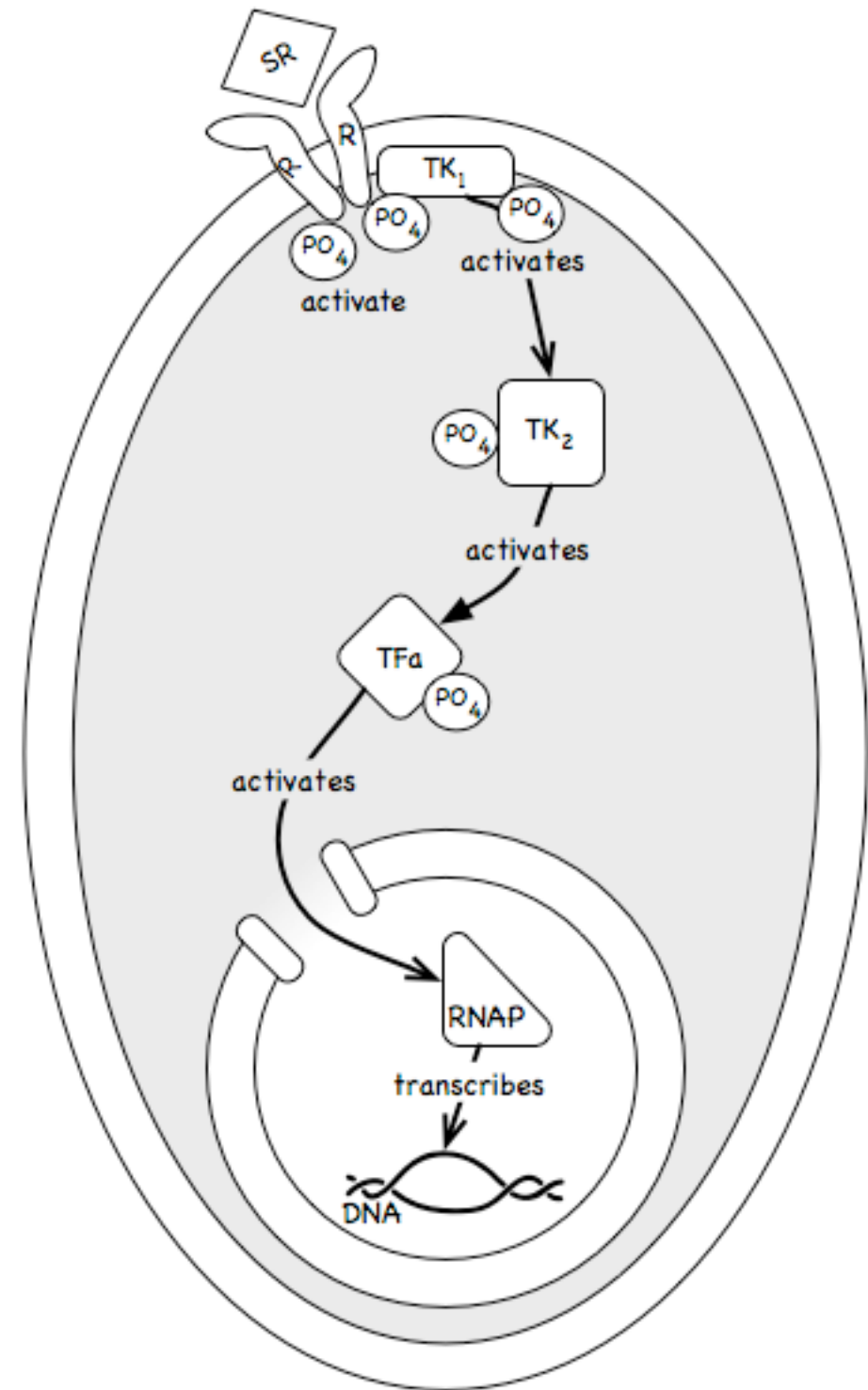
Systems thinking - chunking sub-processes into unified concepts

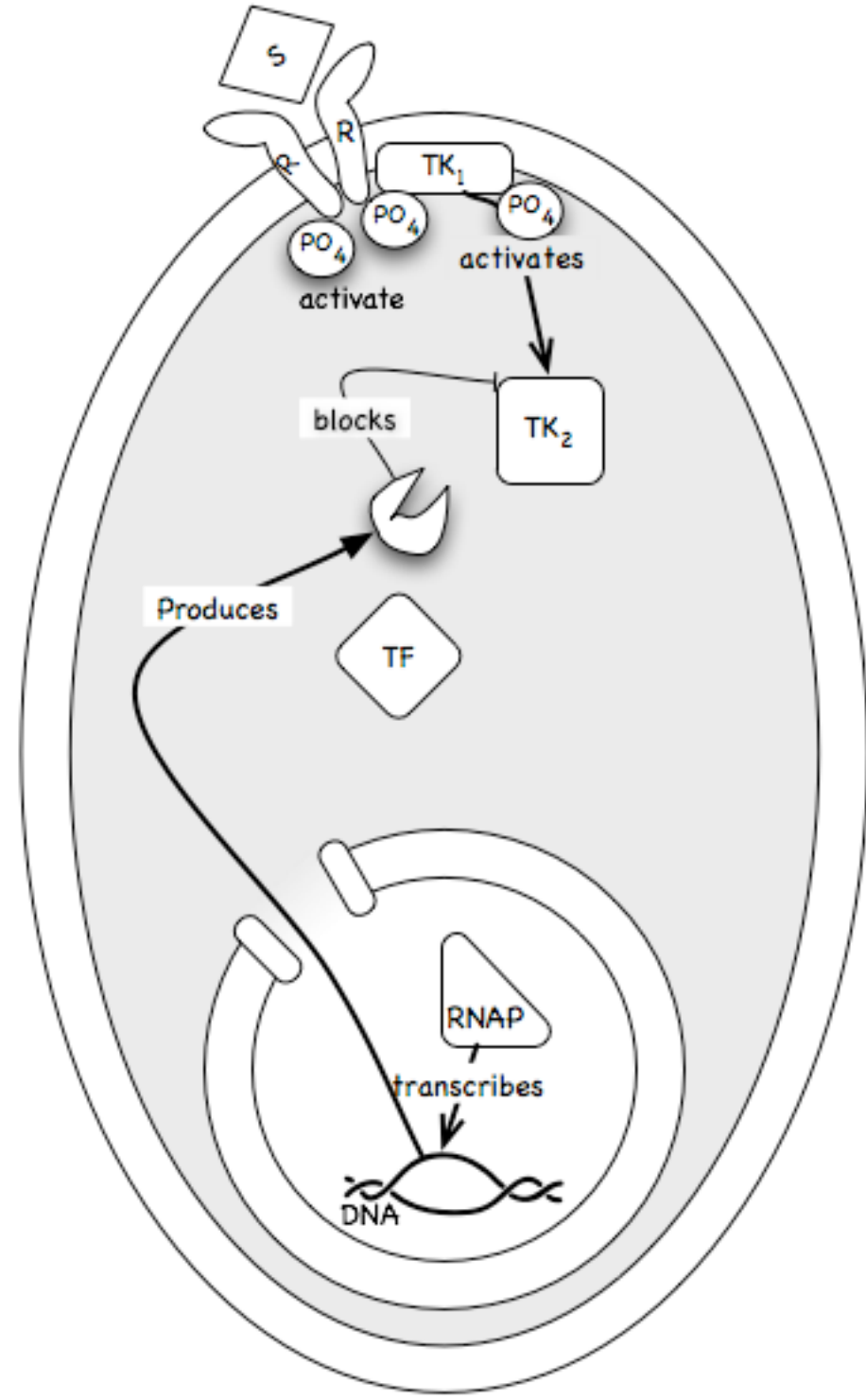
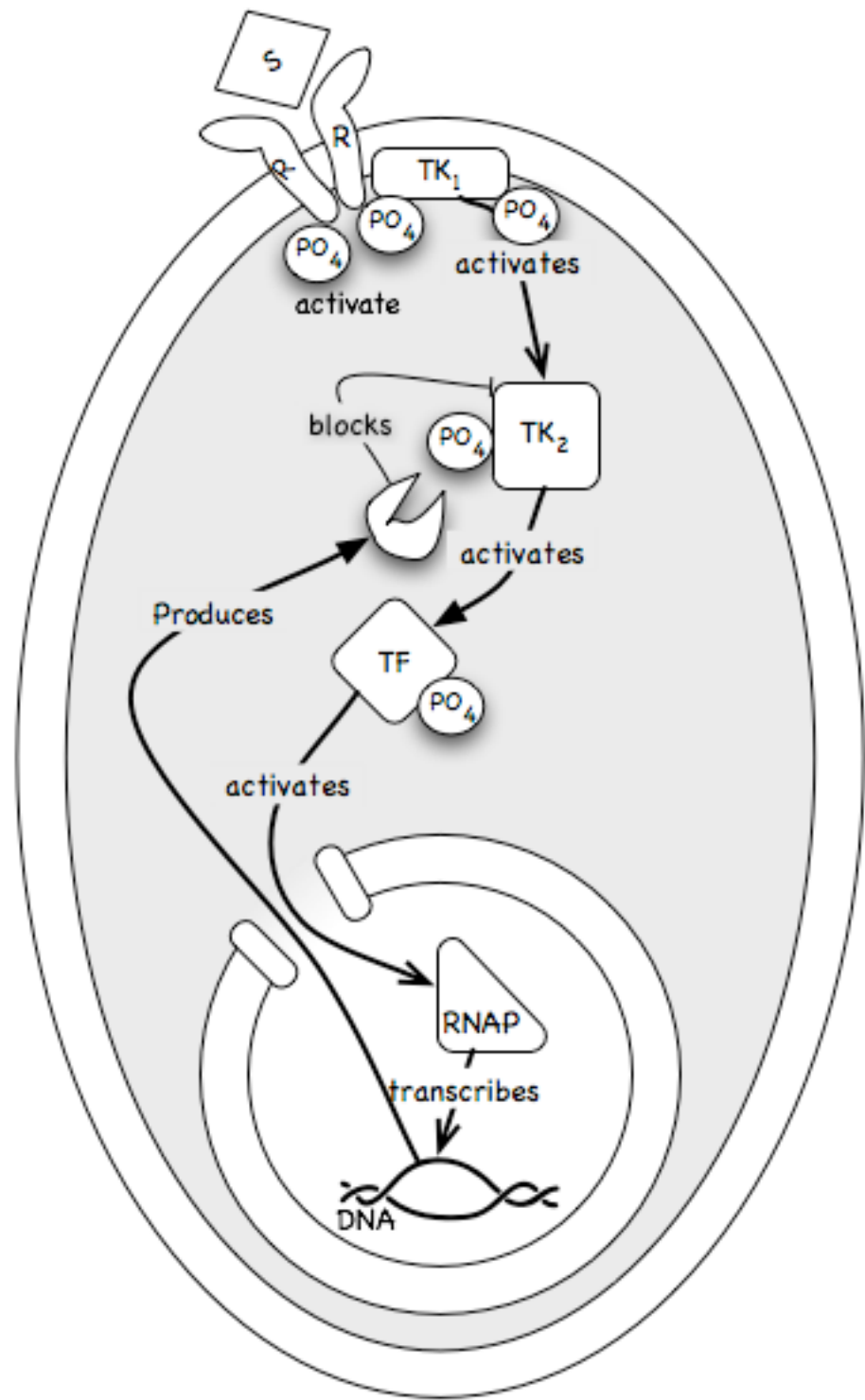
Engage Inductive reasoning to interpret observations in the context of underlying systems.

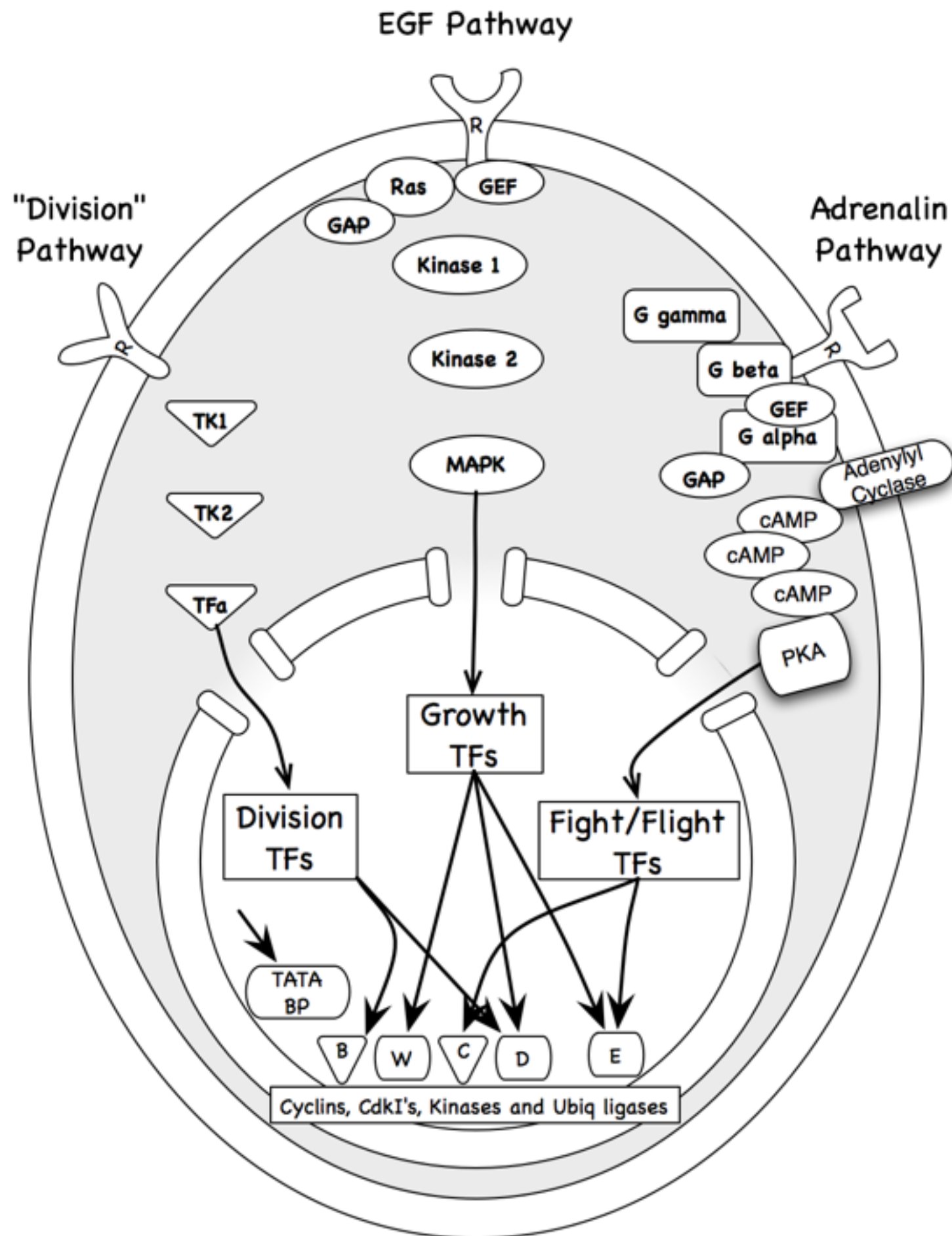
Pathway Off - No Signal Present



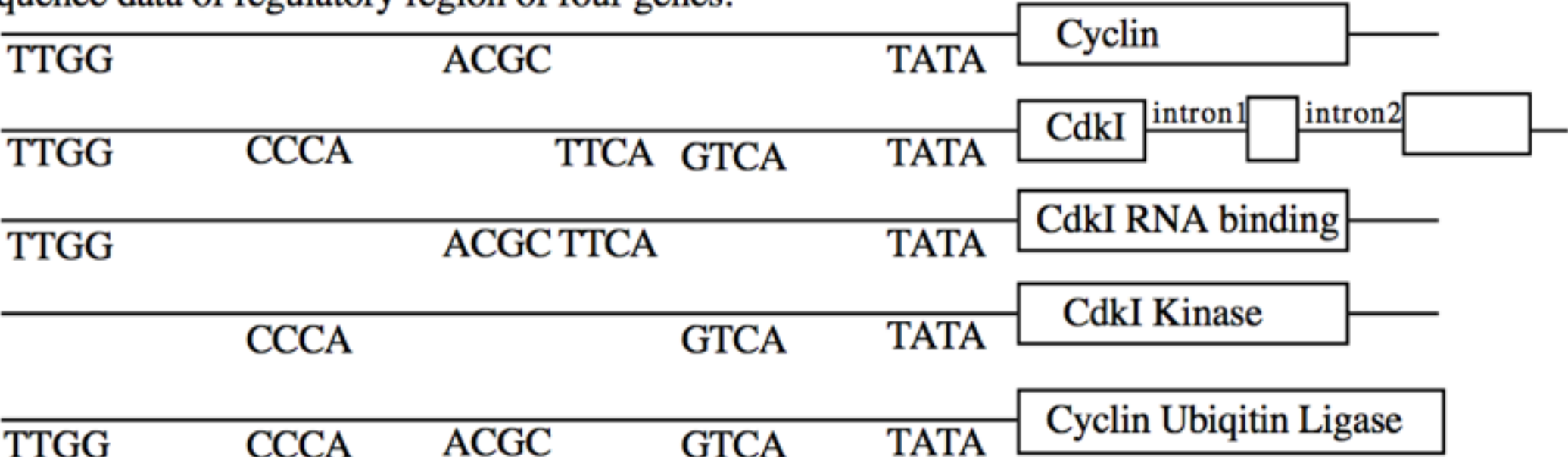
SR Pathway activated by signal binding receptor



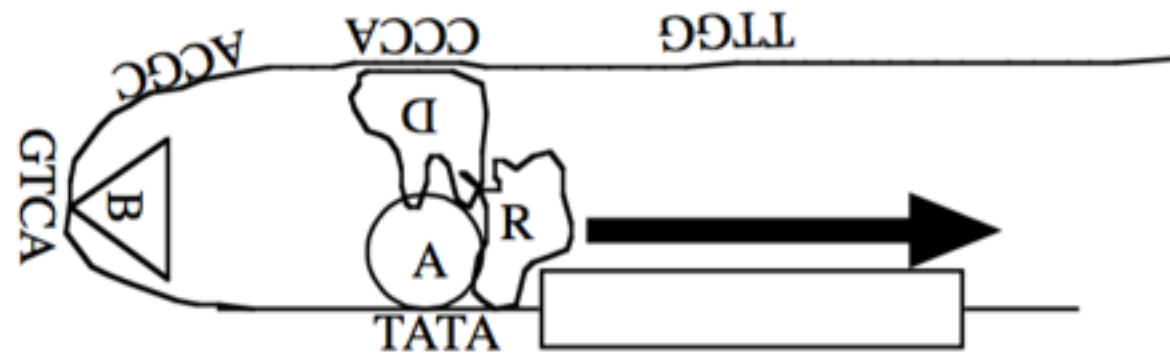




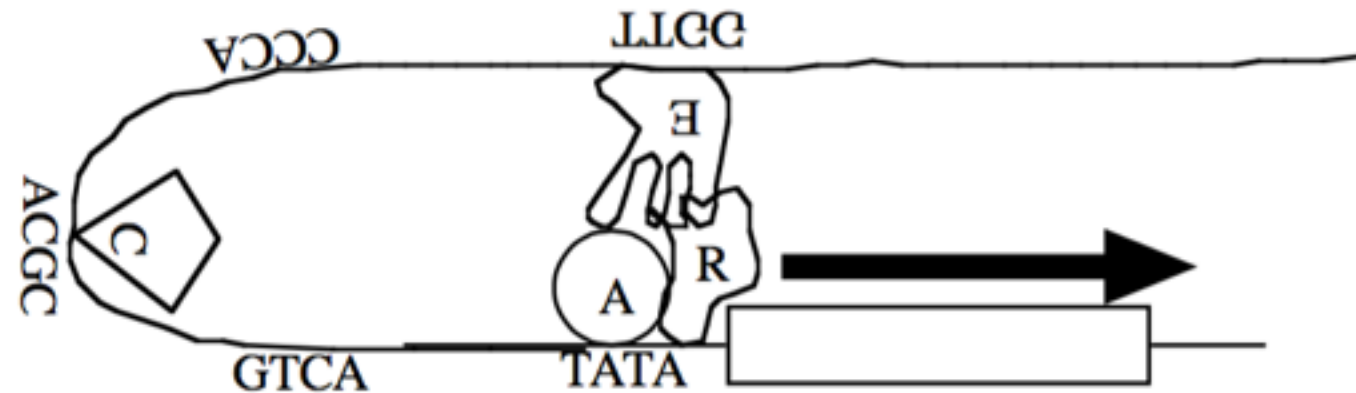
DNA sequence data of regulatory region of four genes.



If Transcription factors A, B and D bind, the gene is transcribed.

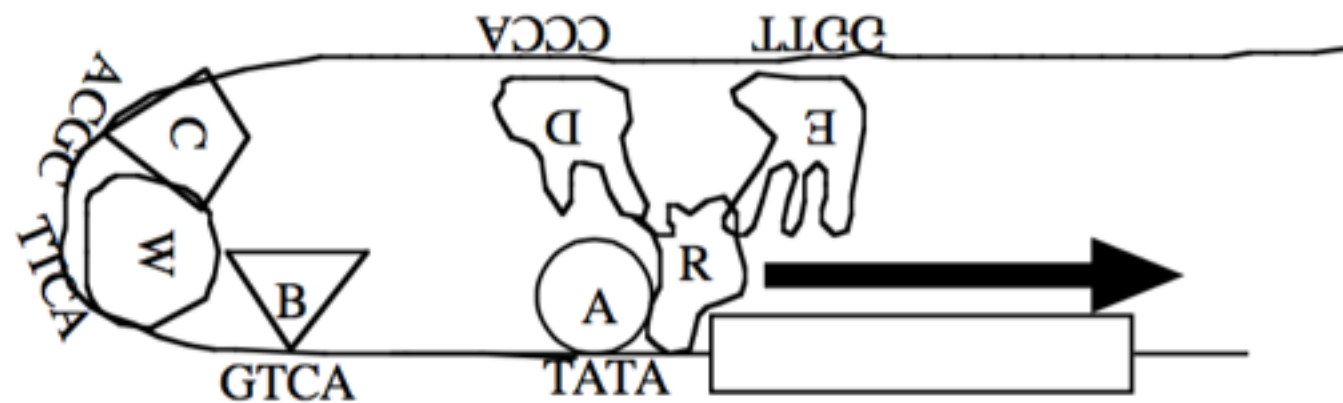


If Transcription factors A, C E bind, the gene will be transcribed.

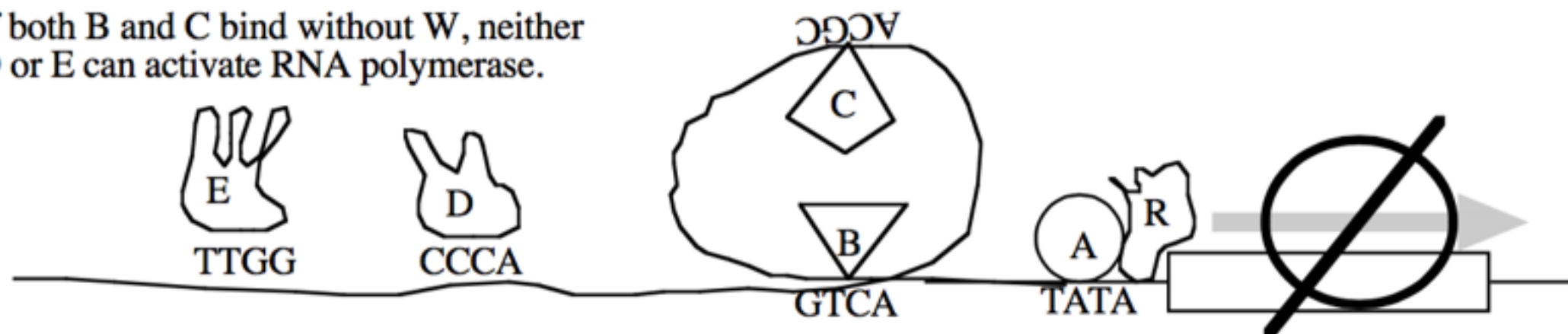


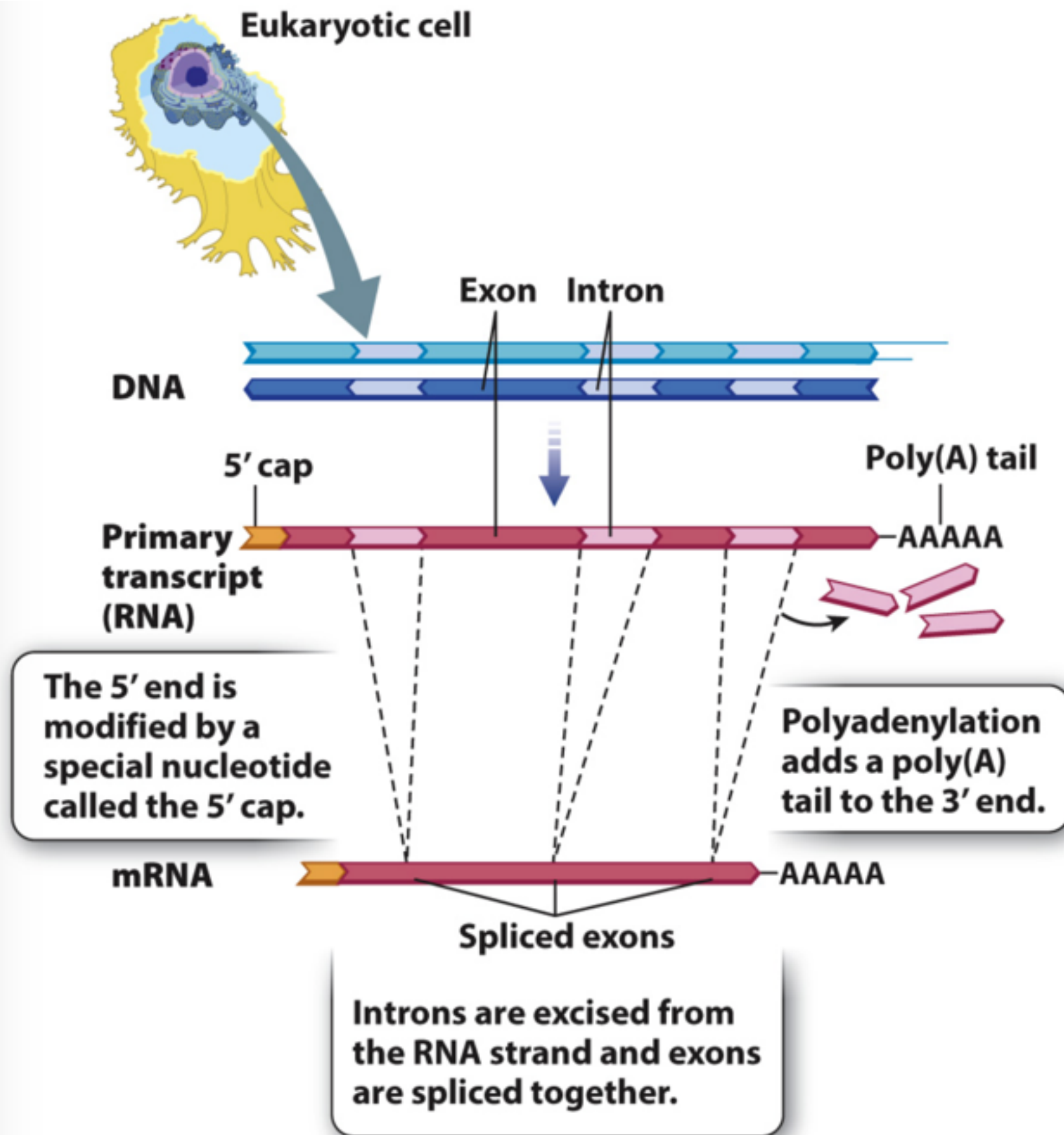
If W binds it changes the way C and B bend and changes the bending to 90°

NOTE: If B, C and W bind, D OR E can activate RNA polymerase.



If both B and C bind without W, neither D or E can activate RNA polymerase.



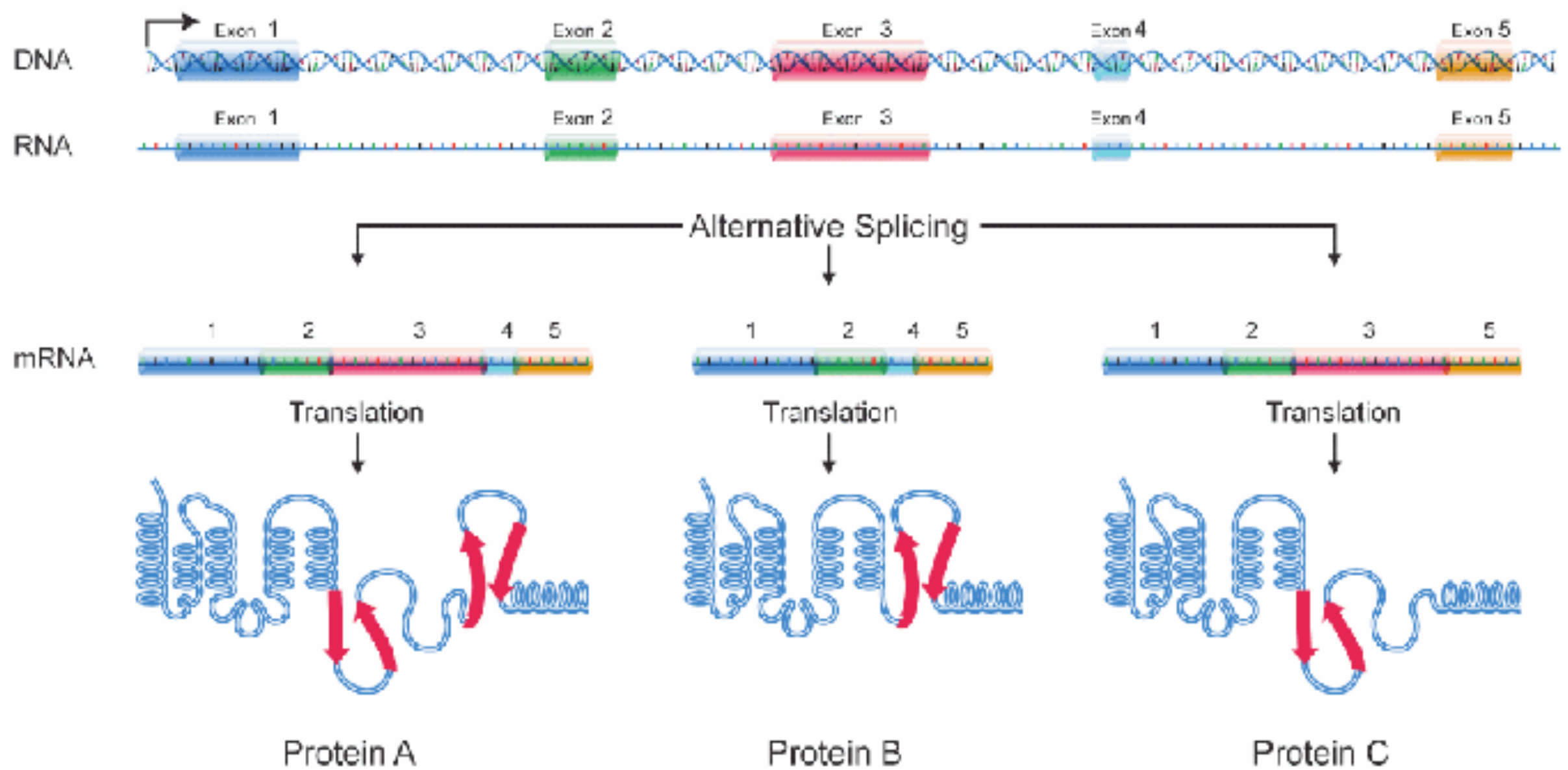


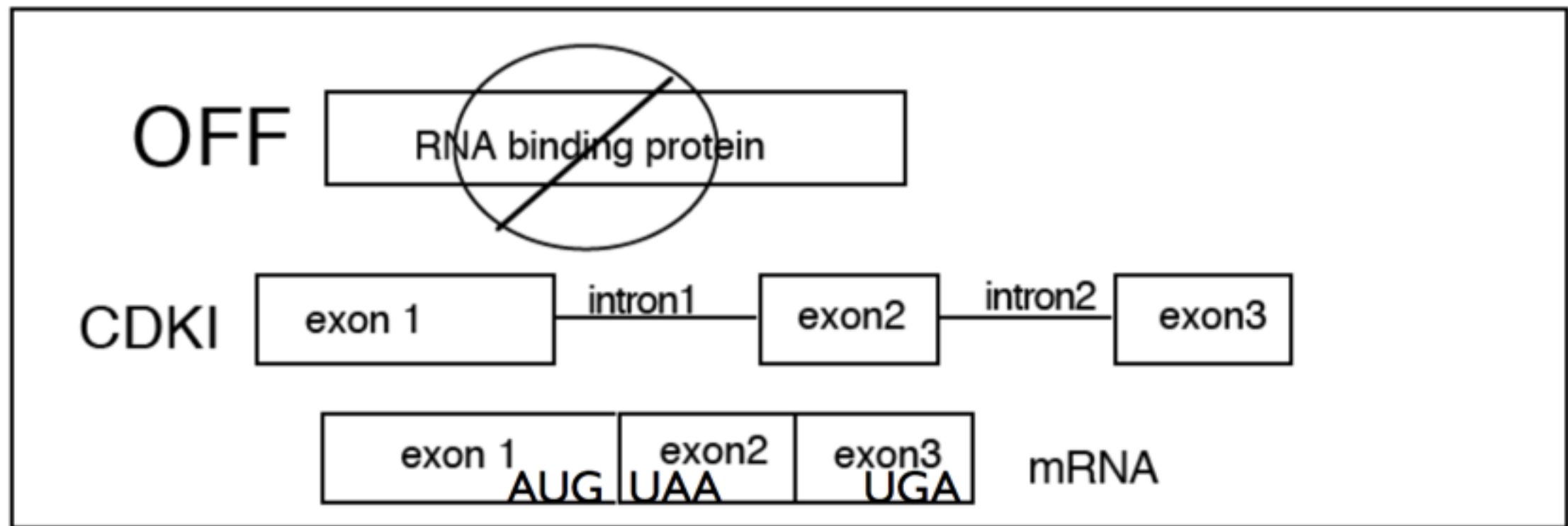
The ends of the 1^o transcript are modified to provide stability.

The 5' end has a 7 methyl Guanine cap.

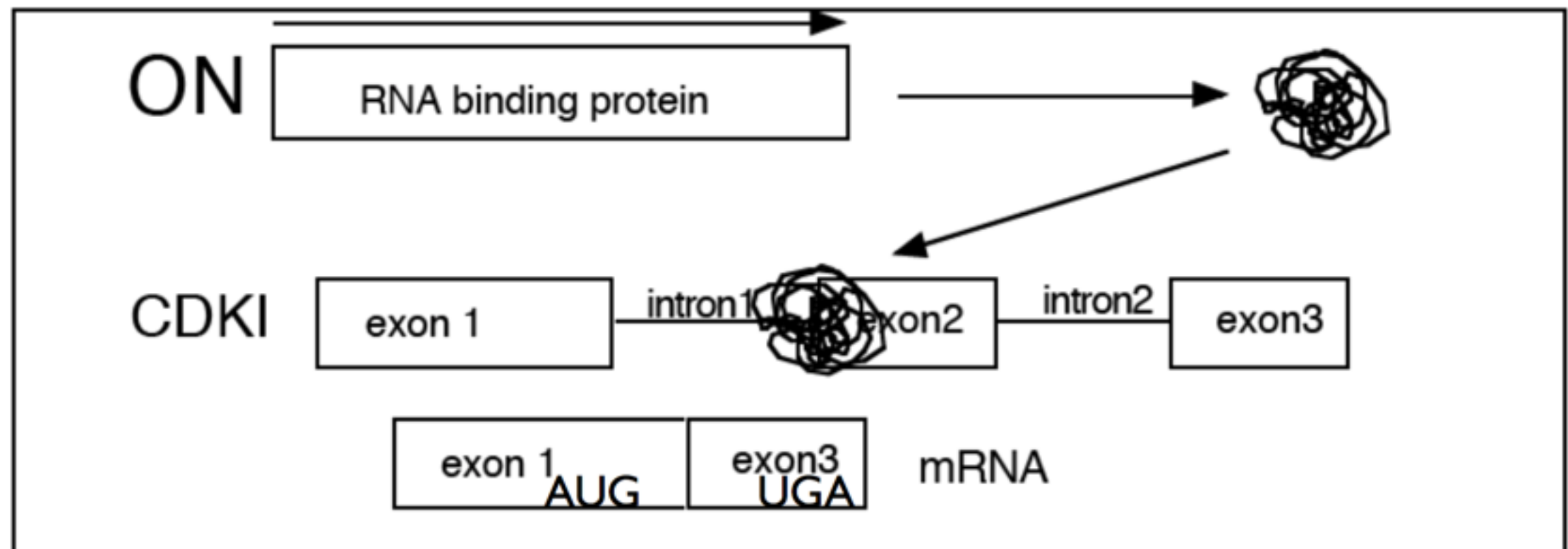
The 3' end has many Adenine nucleotides added to create a “poly A tail”.

FIG. 3.21 Fate of the primary transcript for protein-coding genes in eukaryotes.





RNA binding protein binds to and blocks the acceptor site of intron 1 in the CDKI gene. In RNA processing, the next available acceptor site would be used. The functional CDKI protein requires amino acids encoded in exon 3. The protein produced when exon 2 is present is only 20 amino acids long. The protein produced when exon 2 is absent is 50 amino acids long.



Initiation of Translation

Met-tRNA binds to start with initiation factors.

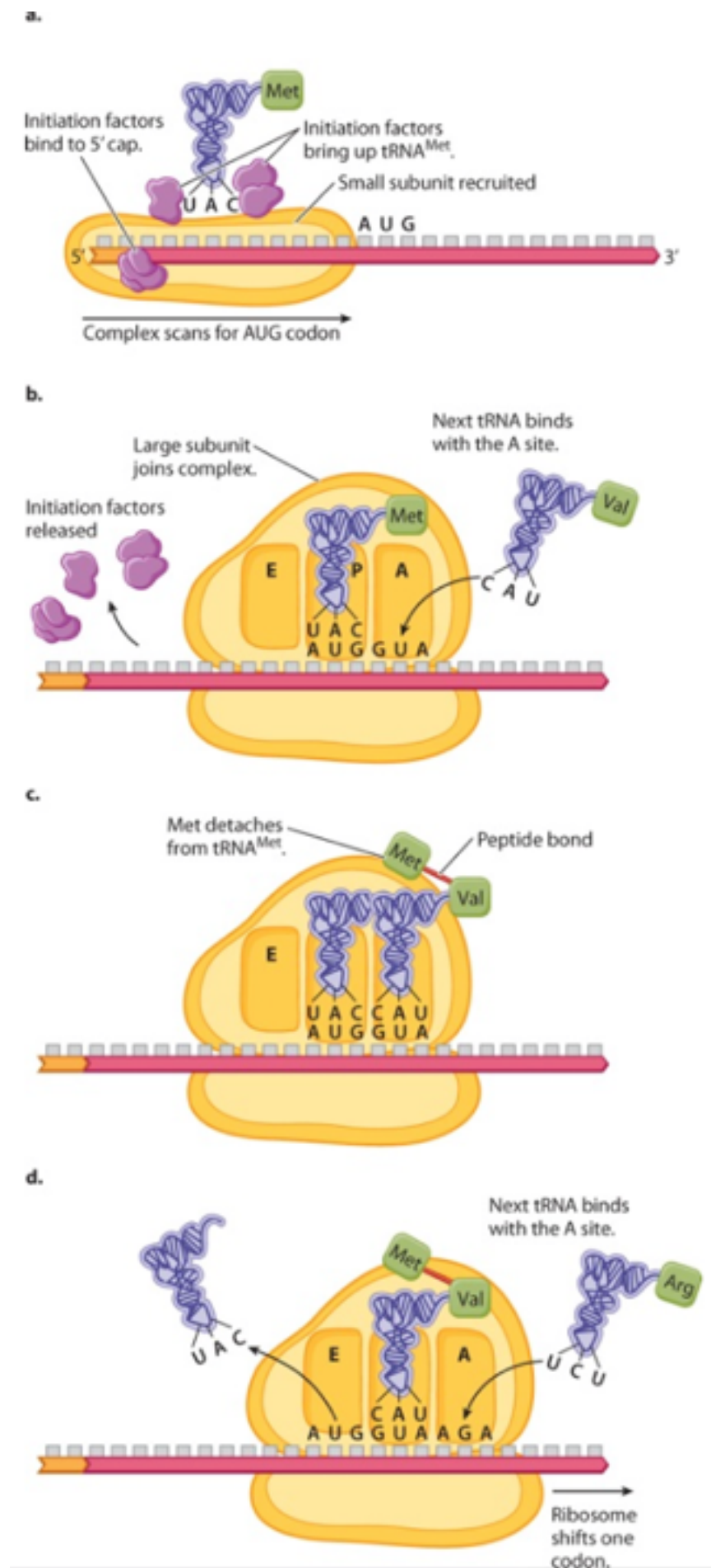
Small subunit binds to mRNA

Large subunit binds
Met-tRNA in P site

Second tRNA binds to A site

First peptide bond forms

Ribosome slides one amino acid
Met-tRNA is released



Only one of the three reading frames encodes the protein.
The open reading frame has a start and stop and the in-frame sequence between that encodes ubiquitin.

Translate Tool - Results of translation

Open reading frames are highlighted in red. Please select one of the following frames - in the next page, you will be able to select your initiator and retrieve your amino acid sequence:

5'3' Frame 1

KS **Stop** RADVRASEKT **Stop** FLNISK SADQK **Stop** RNKLTFVCETIKKVRD **Stop** SPQLKQLEGGSTLSDYNIPFTWSCVVCVV
ACRSSLRPSLARPS PWRSSHPIPSKTSKPRYRTRRKIPQSISV **Stop** SSAESTWRTDALCPTTTTSRRSRPFTWSCVCGV
ACKSS **Stop** RPSLARPS PWRSSHPIPSKTSKPRYRTRRKTPQSISV **Stop** SSAESTWRTDALCPTTTTSRRSRPFTWSCV
VVACRSL **Stop** RPSLARPS PWRSSHPIALKTLRPGSTTRRESPQINSV **Stop** SSPESSWRTDALCPITTSRRNRPFWS
VCVVACRSL **Stop** RPSLARPS PWRLSHPIPSNTSKPGSTTR **Met** ESPQIISV **Stop** FSPESSWRTDVLCPITTTTSRRSRPFTV
TLSGRERKQINPP **Stop** GREQAANFQSTYTYTYKYQRAAVKRIVSCEKVVESGRPSK **Stop** RKQVFEYFKKCRPNVKK
Stop ISFSVRNRKKIP

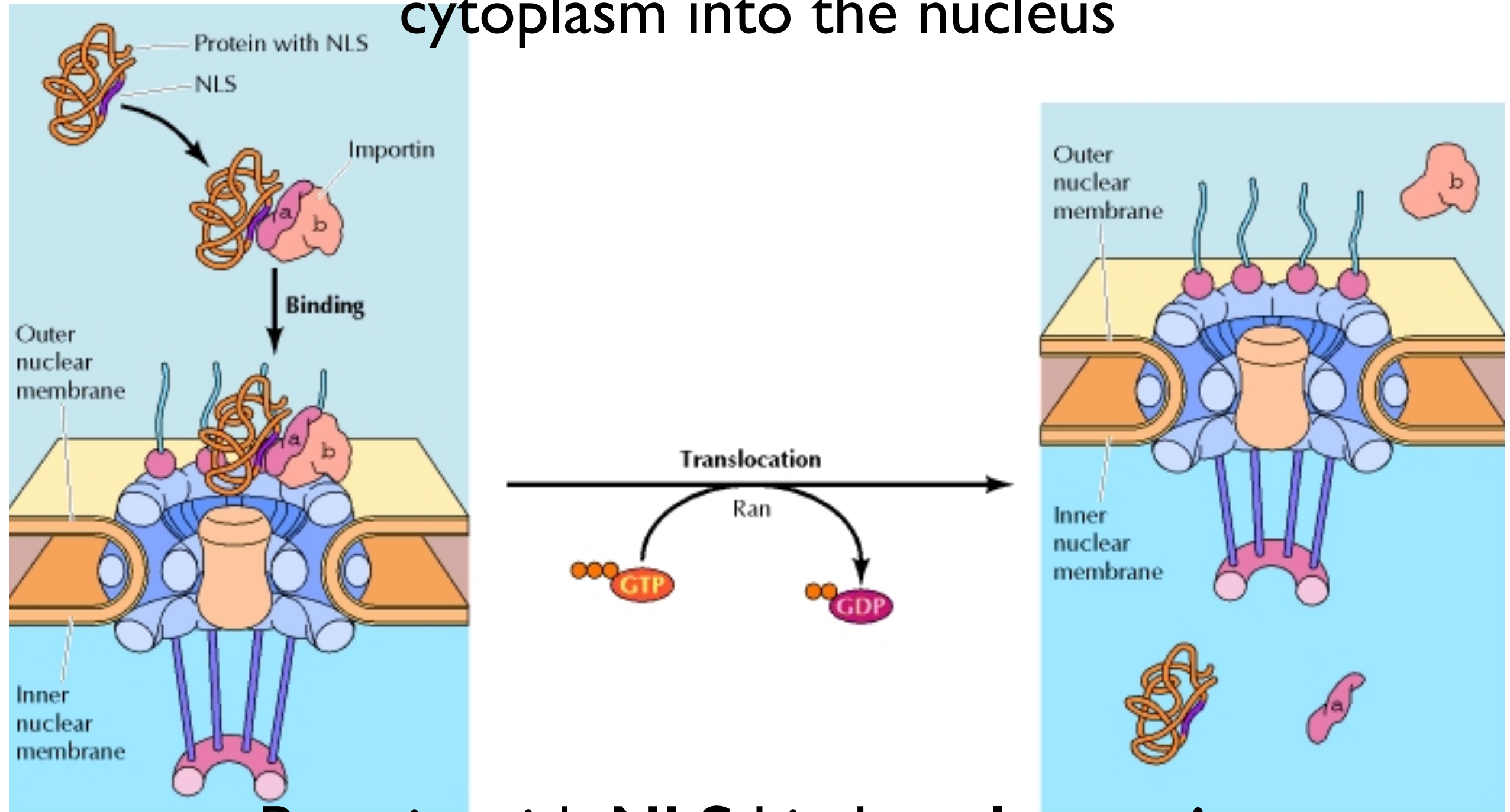
5'3' Frame 2

SRRERTSEQVKKPSF **Stop** IFQKVPTKSKEIN **Stop** LLC AKQ **Stop** KKLEISHHN **Stop** SNWRAEALCLTTTSPSLGPASAWW
HADLR **Stop** DPHWQDHHLGGRAIRYHRKRQSQDTGQGGKSPRASAFDLRRKAPGERTHSVRLQHPEGVDHLLGPASA
GWHANLREDPHWQDHHLGGRAIRYHRKRQSQDTGQGGKPPRASAFDLRRKAPGERTHSVRLQHPEGVDHLLGPAS
AWWHADLCEDPHWQDHHLGGRAIR **Stop** H **Stop** KR **Stop** GQDPRQG GGNPPRSTAFDLRRKAVGGRTHSVRLQHPEGIDP
SLGPASAWWHADLCEDPHWQDHHLGG **Stop** AIRYHQTRQSQDPRQGWNPPRSSAFDFRRKAAGGRTYSVRLQHPEG
VDPSQSL **Stop** VVESENKLTHREGESRRRISSQLIPINTSALL **Stop** NES **Stop** AVKKS **Stop** RADVRASEESKFLNISK SAD
Q **Met** **Stop** RNKLAFLCETGKKF

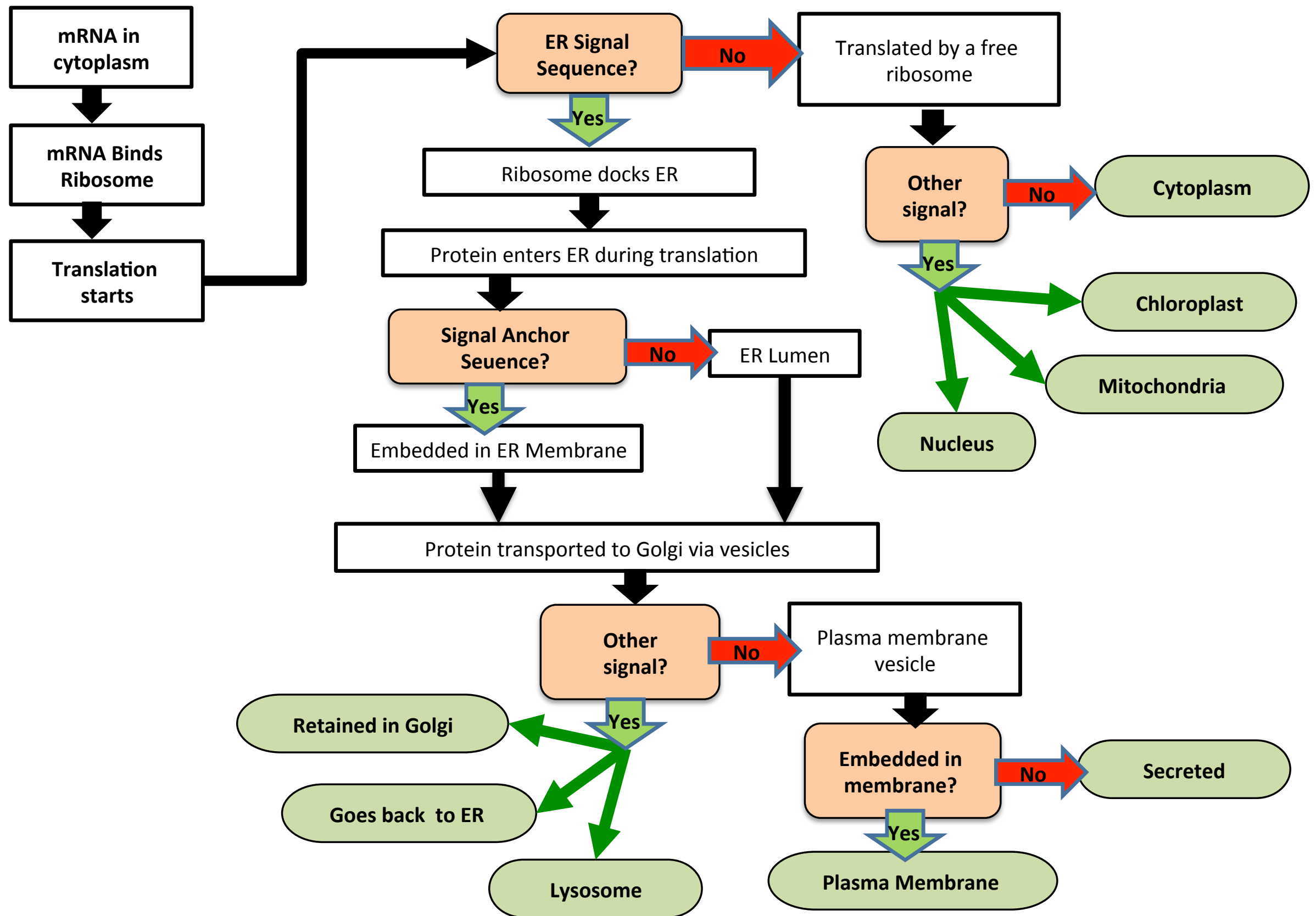
5'3' Frame 3

VVESGRPSK **Stop** KNLVFEYFKKCRPKVKK **Stop** TNFCVRNNKKS **Stop** RLVT TTKATGGRKHSV **Stop** LQHPLHLVLRRLRG
G **Met** QIFVKT LTGKTITLEVEPSDTIENVKAKIQDKEENPPEHQRLIFGGKHLENGRTLSDYNIQKESTIYLVLRRLRGG
Met QIFVKT LTGKTITLEVEPSDTIENVKAKIQDKEENPPEHQRLIFGGKHLENGRTLSDYNIQKESTIYLVLRRLRGG **Met**
QIFVKT LTGKTITLEVEPSDSIENVKARIHDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG **Met** QIF
VKT LTGKTITLEVEPSDTIKHV KARIHDKDGI PPDHQRLIFAGKQLEDGRTLSDYNIQKESTLHSHFKW **Stop** RAKTN **Stop**
PTVRERAGGEFPVNLYLYL **Stop** IPARCCKTNRKL **Stop** KSRREERTSEQVKKASF **Stop** IFQKVQTKCKEIN **Stop** LFC AKQEK
NS

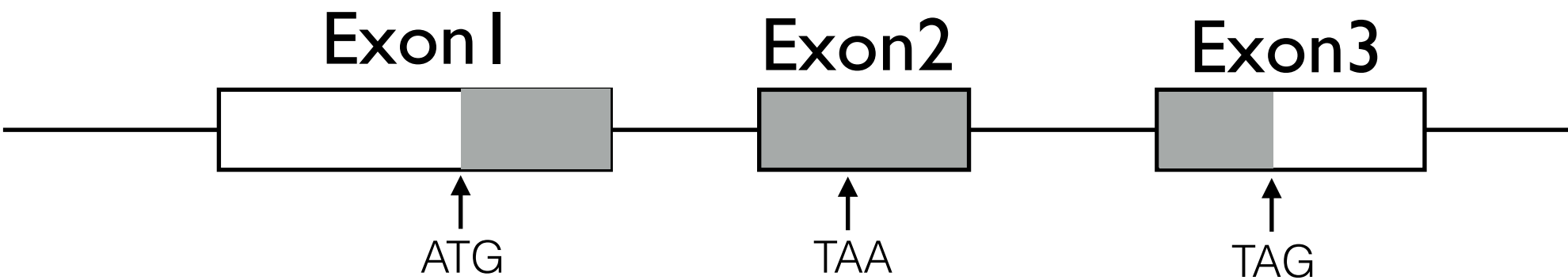
Nuclear Localization Signal (NLS) required to move proteins from the cytoplasm into the nucleus



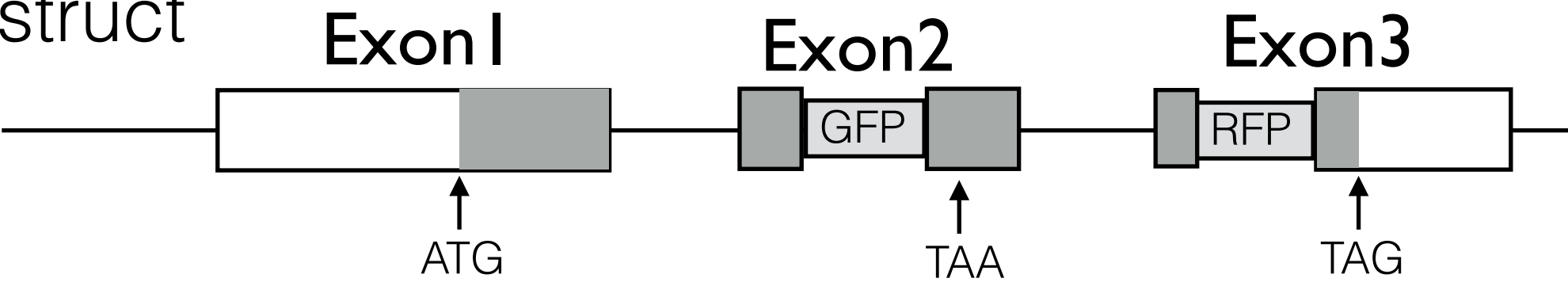
Protein with NLS binds to Importin.
Importin interacts with Nuclear Pore Complex
and moves into nucleus



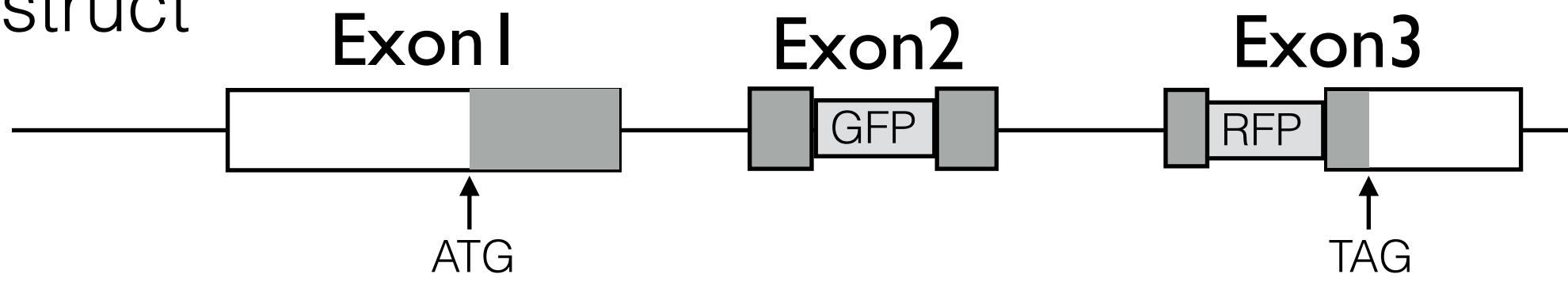
Normal CDKI gene



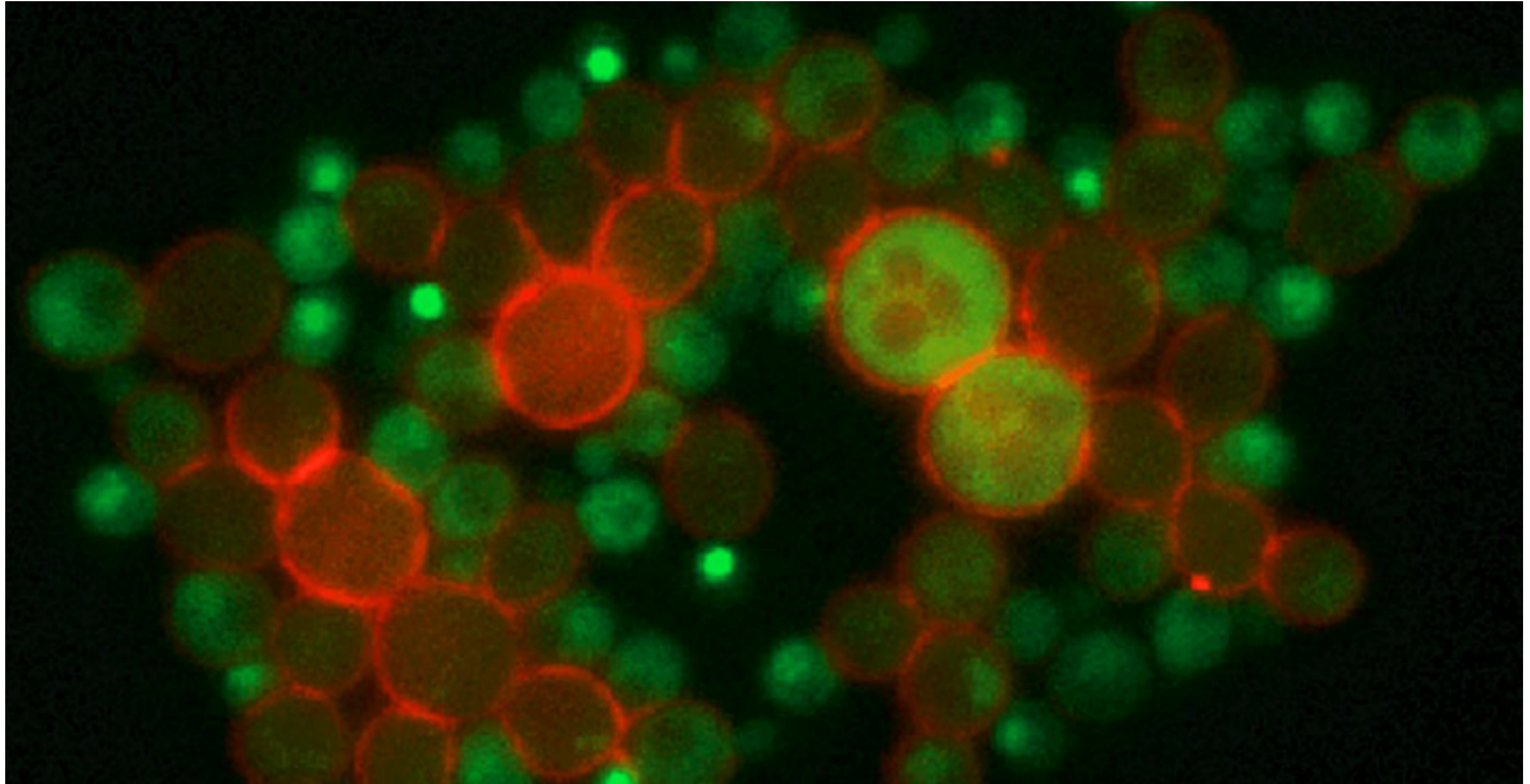
Keisha's construct



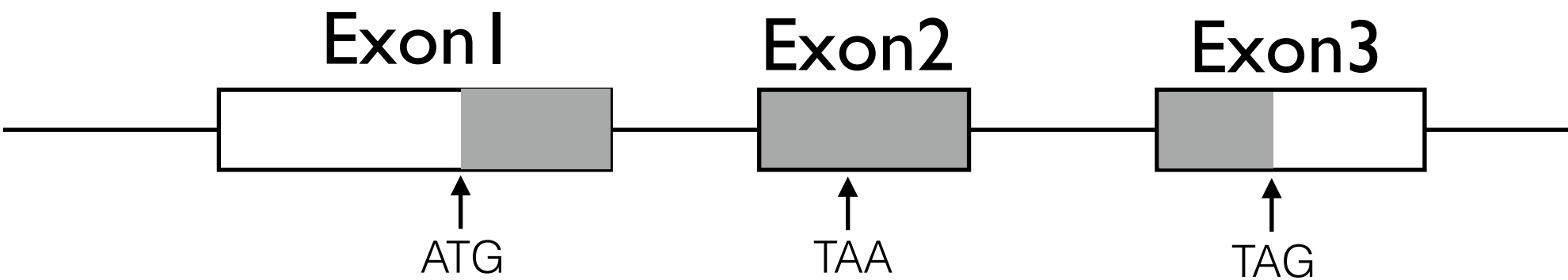
Matt's construct



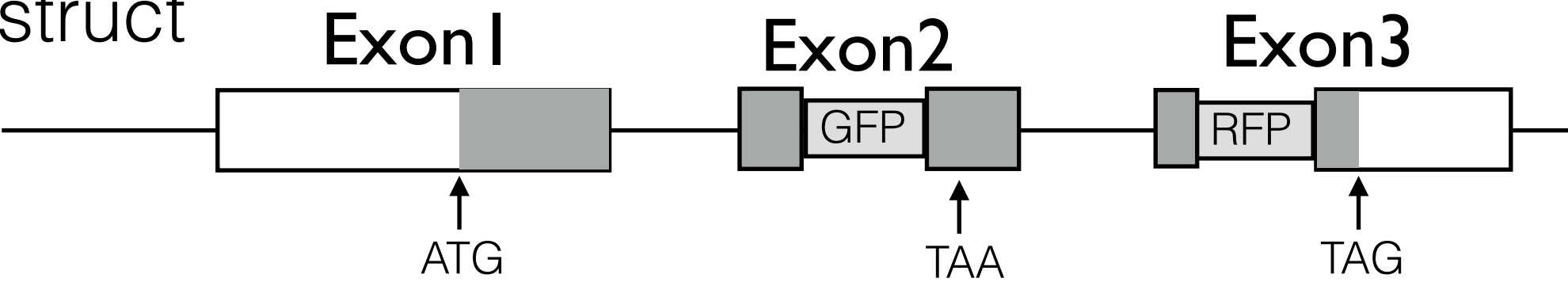
GFP - Green Fluorescent Protein
RFP - Red Fluorescent Protein



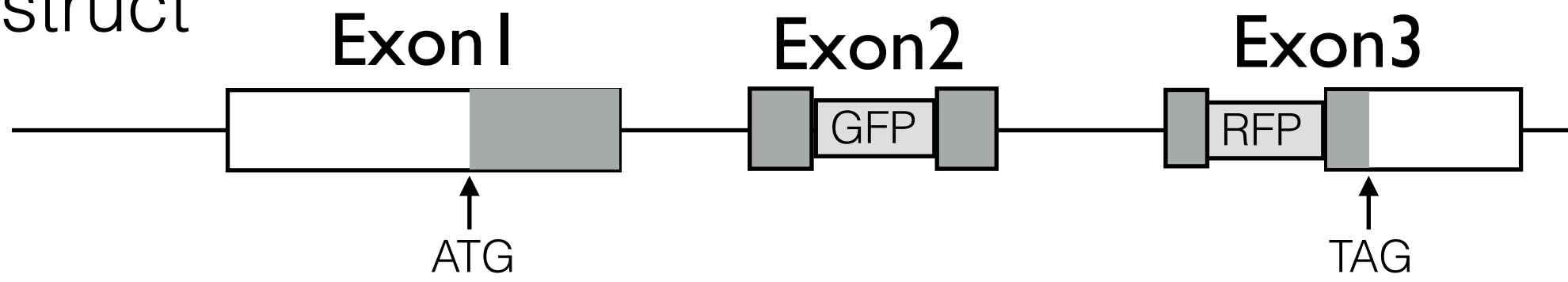
Normal CDKI gene

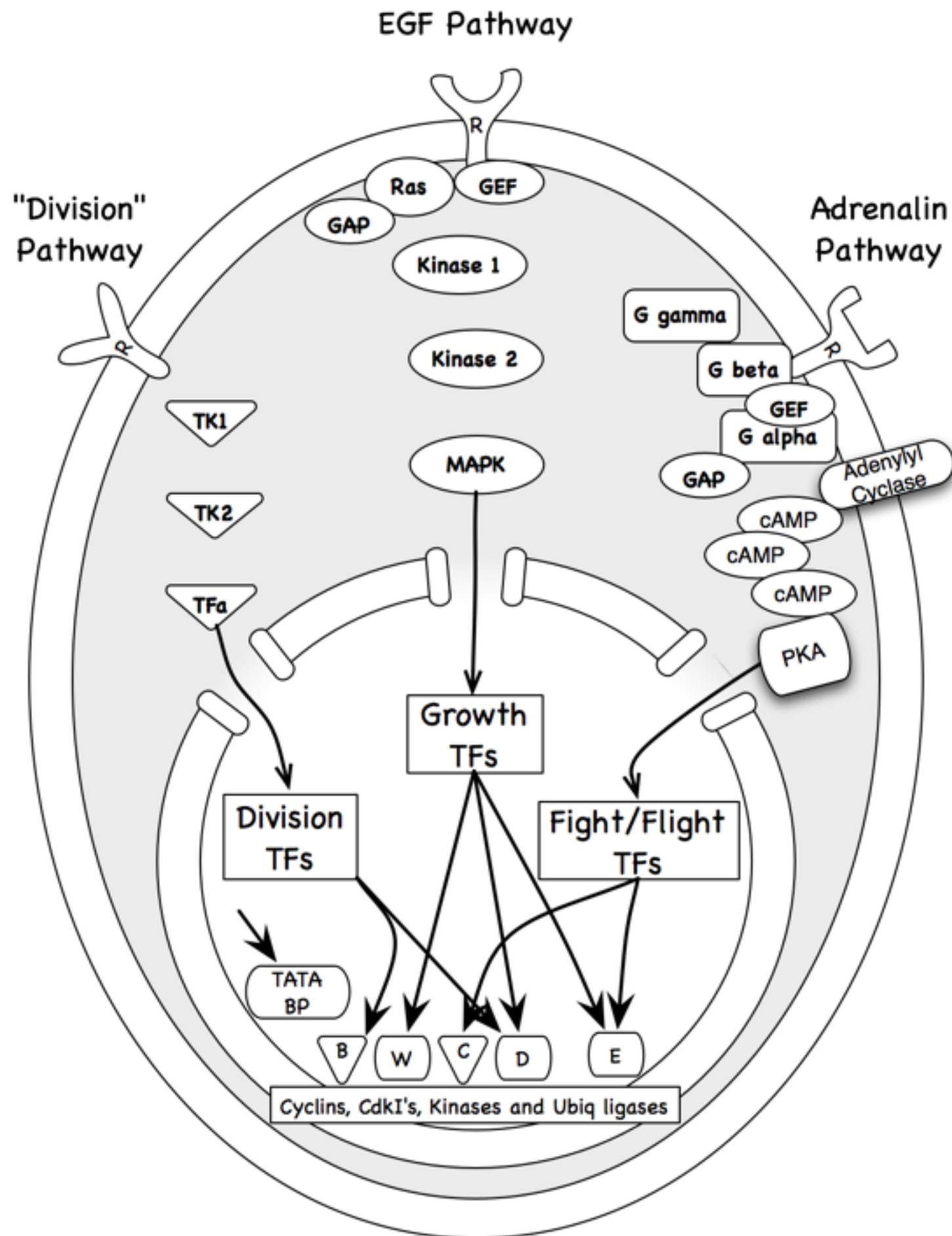


Keisha's construct

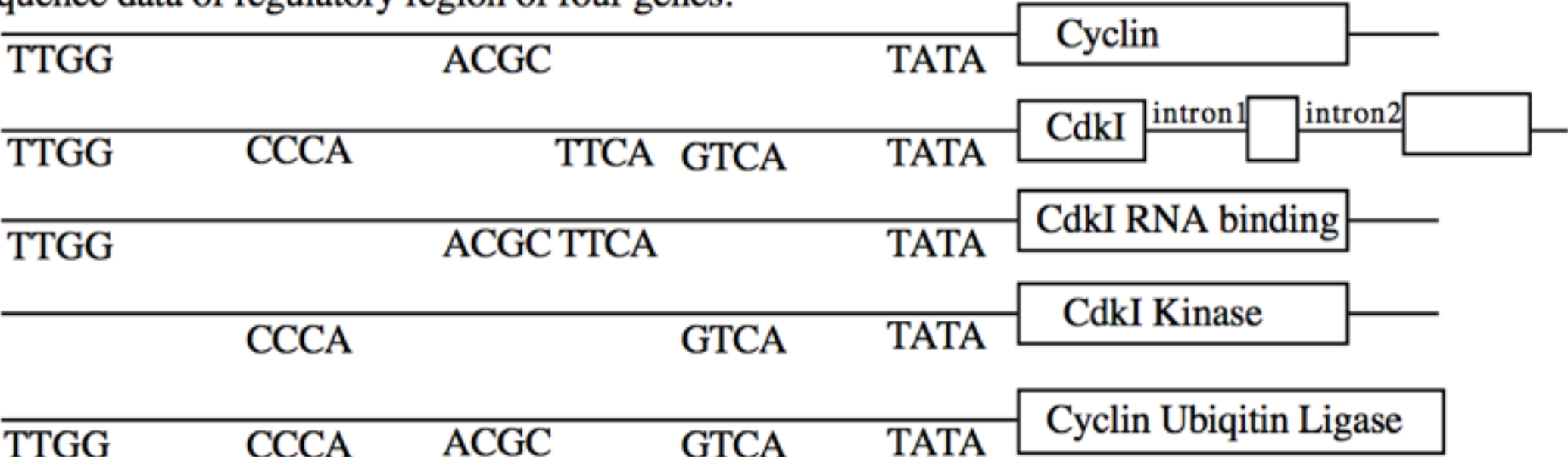


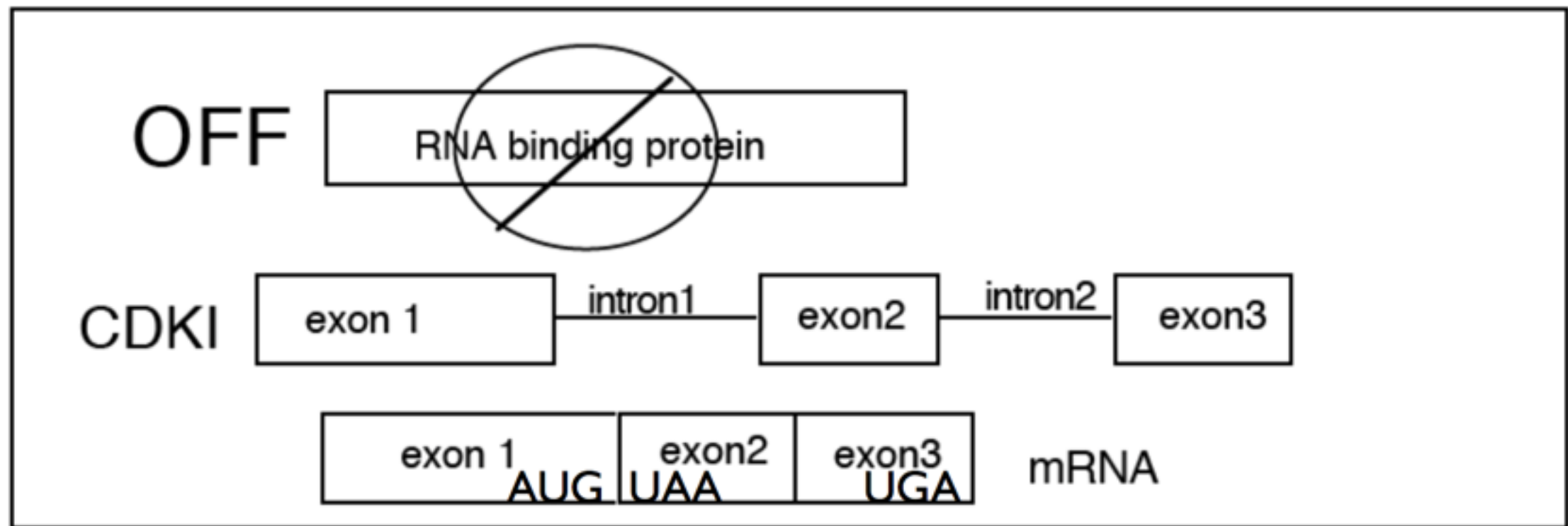
Matt's construct



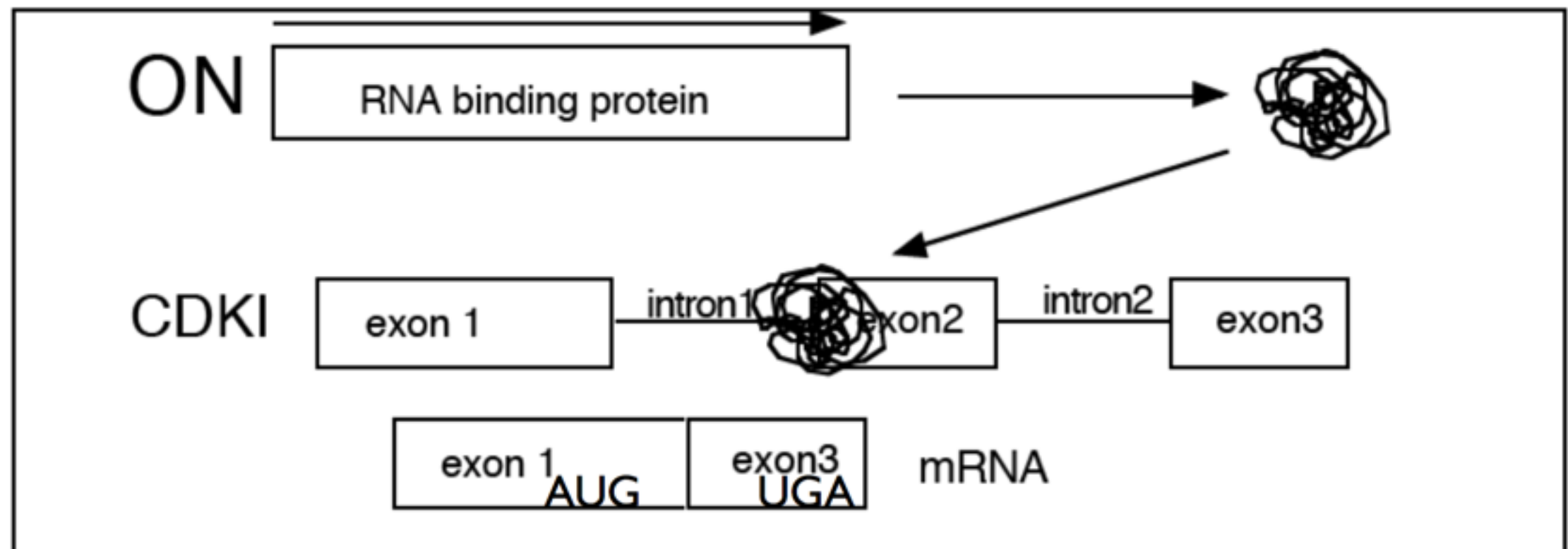


DNA sequence data of regulatory region of four genes.



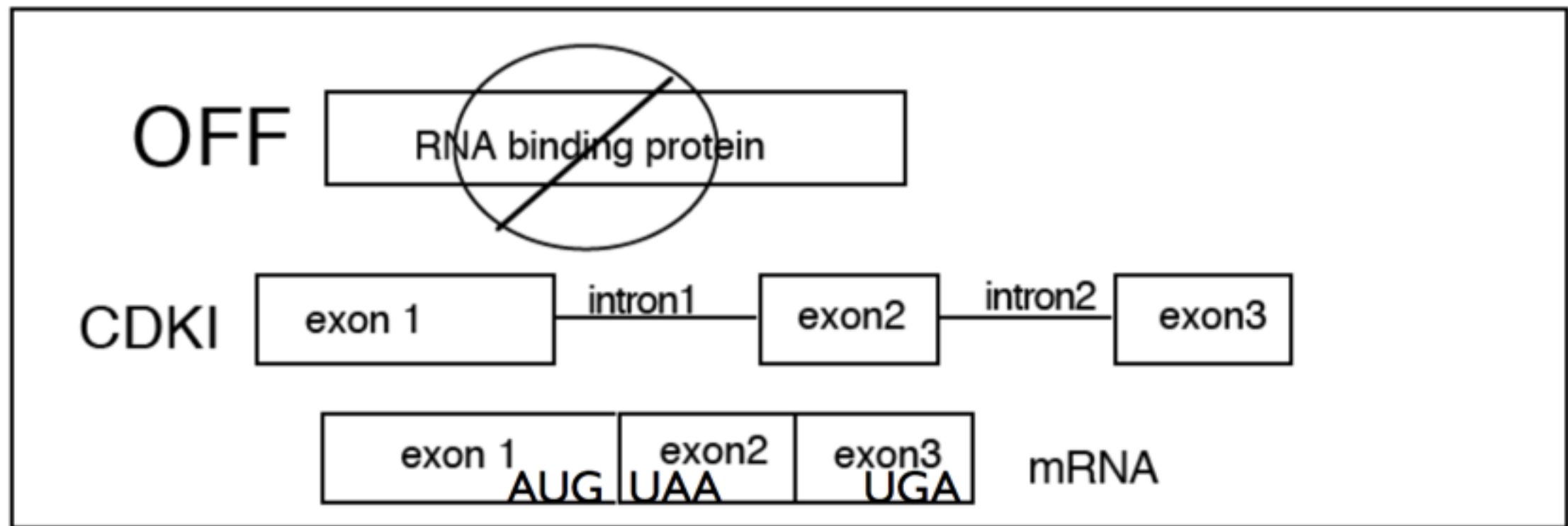


RNA binding protein binds to and blocks the acceptor site of intron 1 in the CDKI gene. In RNA processing, the next available acceptor site would be used. The functional CDKI protein requires amino acids encoded in exon 3. The protein produced when exon 2 is present is only 20 amino acids long. The protein produced when exon 2 is absent is 50 amino acids long.

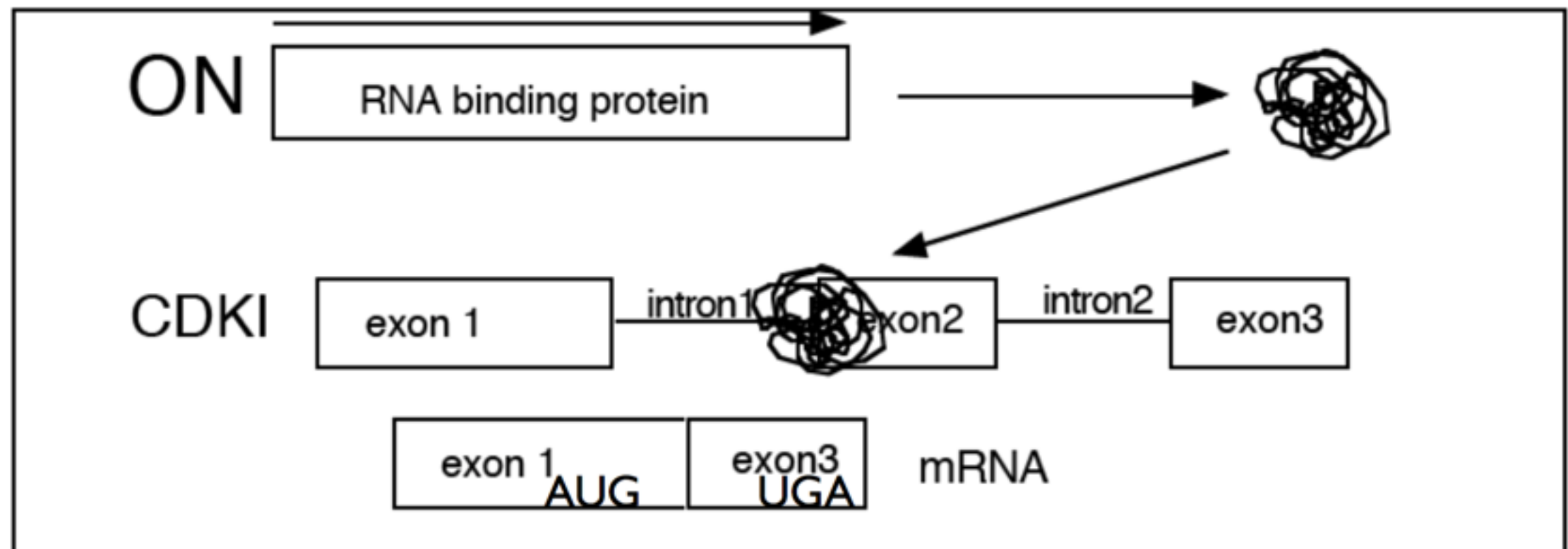


The following summarize the regulation of the CDKI

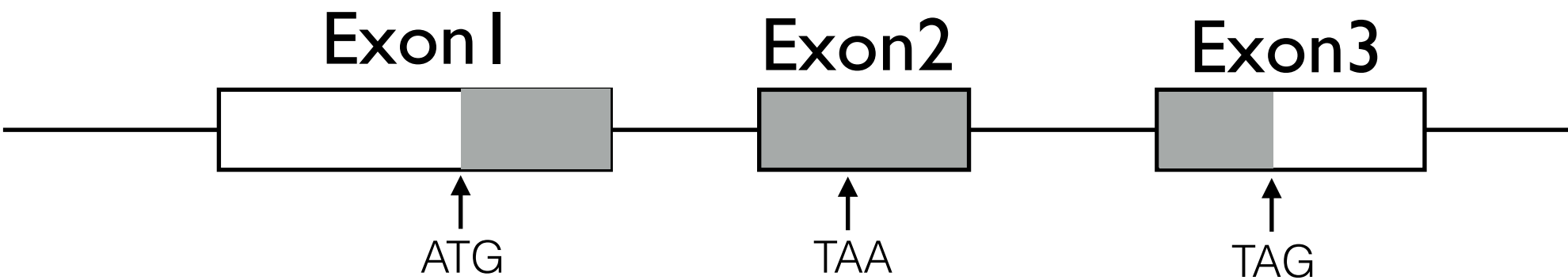
- **Transcription** of the CDKI gene is controlled by transcription factors **regulated by cell signaling**.
- **Processing** of the CDKI mRNA is regulated by RNA binding protein, transcription of which **is also regulated by cell signaling**.
- When **only** the **Division** signal is present, **only** transcription of the **CDKI gene** is activated.
- When **Division and Adrenalin** signals are both present, transcription of both **CDKI and the RNA binding protein** genes is activated.
- When both genes are transcribed, the **RNA binding protein** blocks the acceptor site of Intron 1 of the CDKI gene, **causing Exon 2 to be excluded from the CDKI mRNA**.



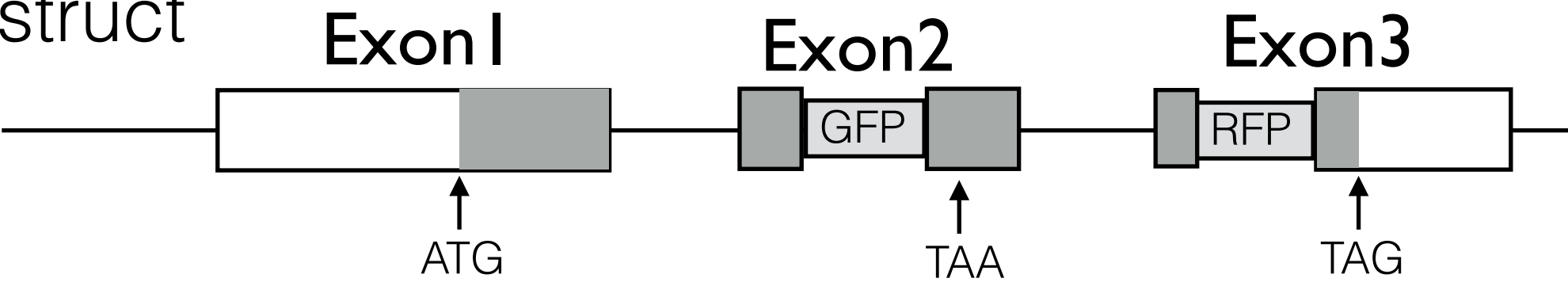
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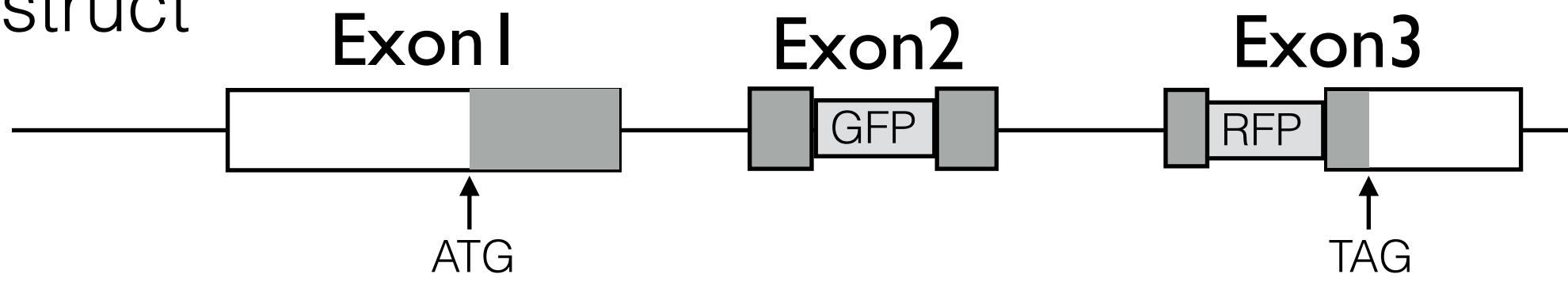
Normal CDKI gene



Keisha's construct

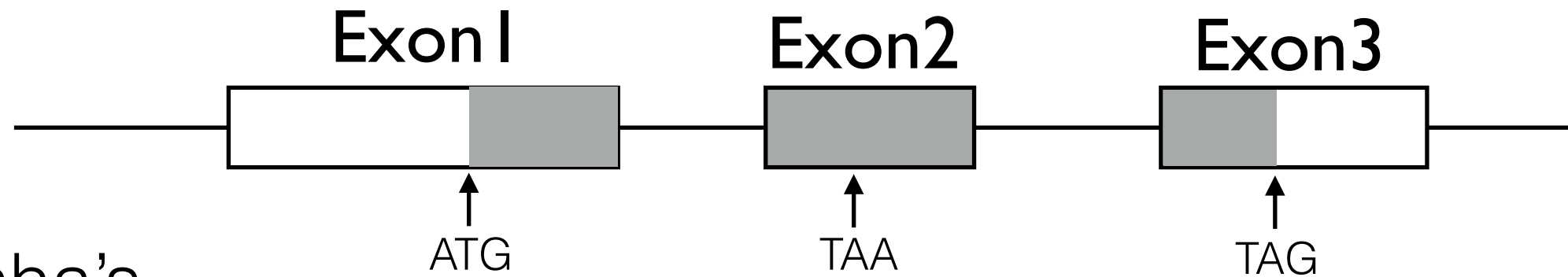


Matt's construct

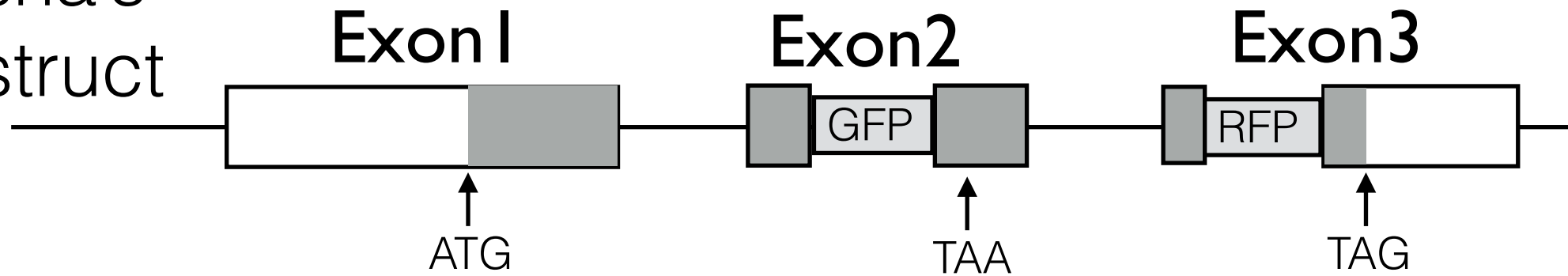


Solution Diagram (not shown to students)

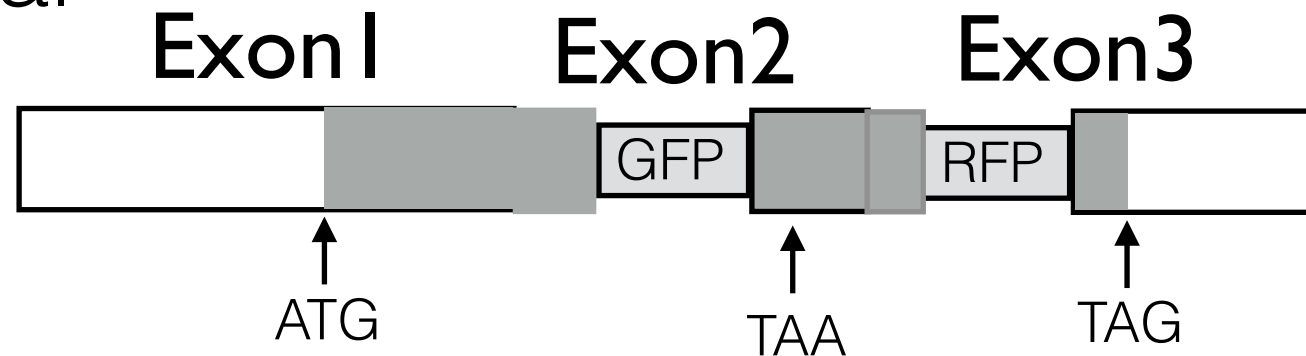
Normal CDKI gene



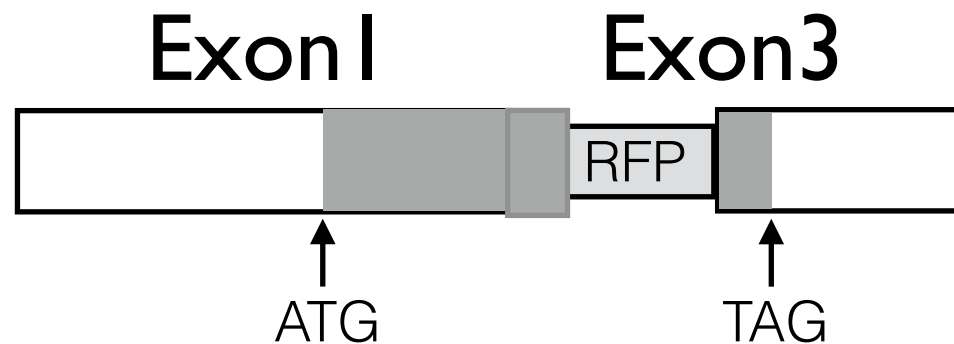
Keisha's construct



Division signal only



Division and Adrenalin



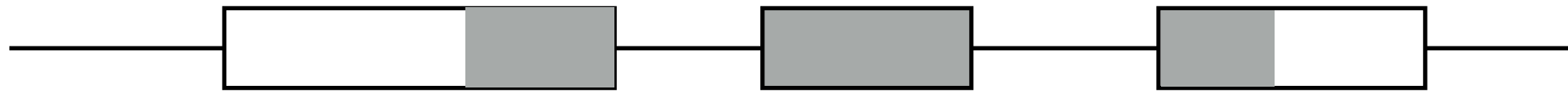
Solution Diagram (not shown to students)

Normal CDKI gene

Exon 1

Exon 2

Exon 3



↑
ATG

↑
TAA

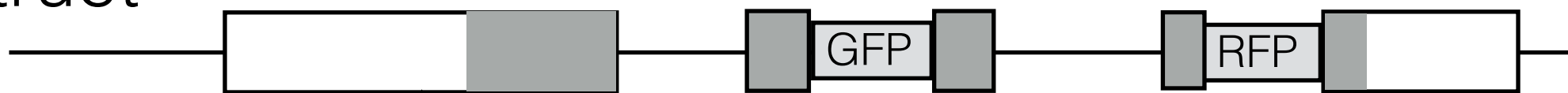
↑
TAG

Matt's
construct

Exon 1

Exon 2

Exon 3



↑
ATG

↑
TAG

Division
Only

Exon 1

Exon 2

Exon 3



↑
ATG

↑
TAG

Division
and
Adrenalin

Exon 1

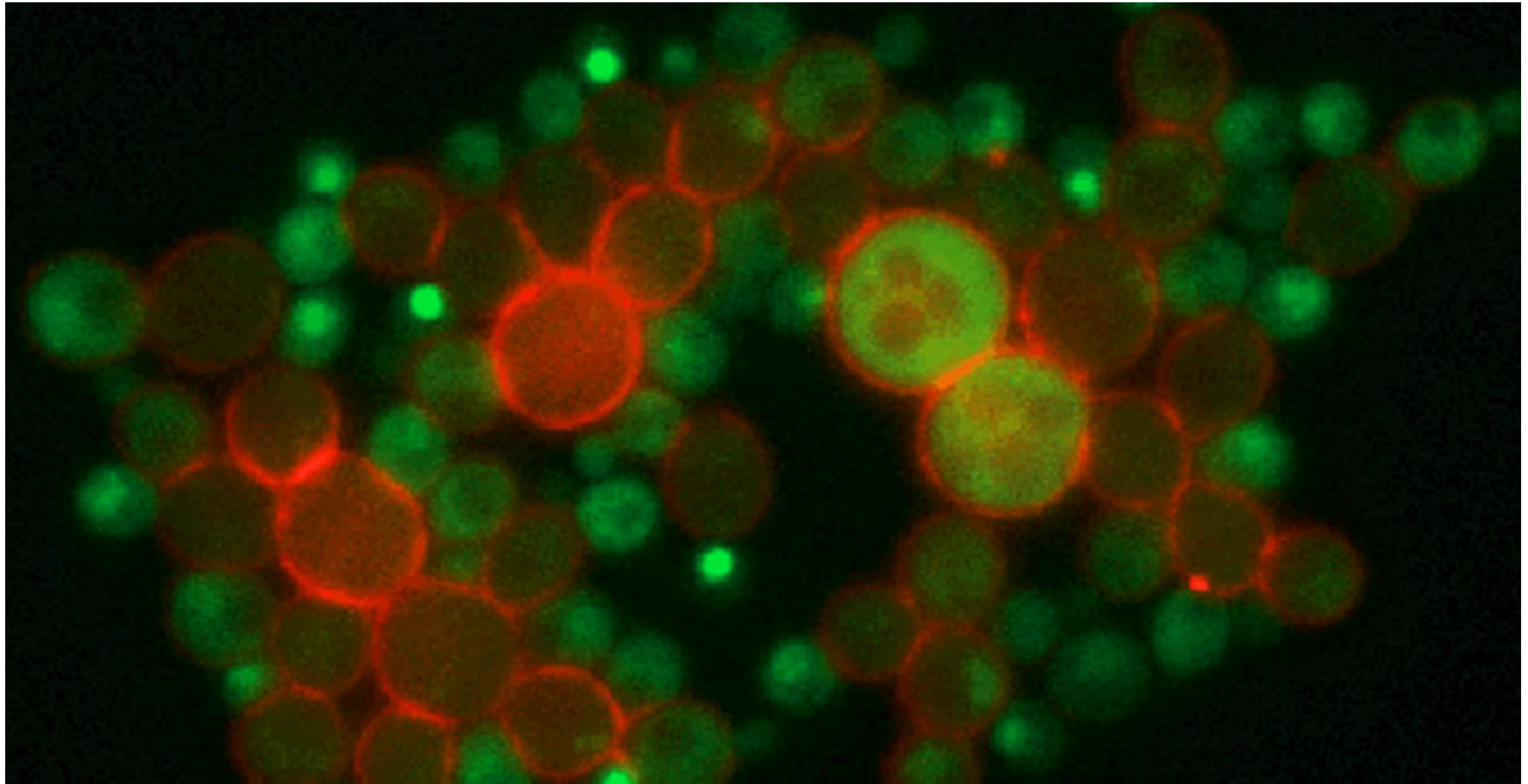
Exon 3



↑
ATG

↑
TAG

GFP - Green Fluorescent Protein
RFP - Red Fluorescent Protein



The following questions use the diagrams of Keisha and Matt's constructs

Problem [32-37] These problems provide a Result, and describe which Construct was used.

Your job is to determine whether the Interpretation properly predicts the Results, given the construct used.

If they do, it is True if they don't it is False.

	<u>Results</u>	<u>Construct(s)</u> <u>Used</u>	<u>Interpretation</u>
T32.	The nucleus glows red and green.	Matt's Construct	The cell was exposed to only the Division Signal. A NLS is encoded in Exon3 of the CKDI gene.
F33.	The cytoplasm glows green and the nucleus glows red.	Matt's Construct	The cell was exposed to only the Division Signal. The NLS is encoded in Exon 3 of the CKDI gene.
F34.	The cytoplasm glows red and the nucleus glows green and red.	Keisha's Construct and Matt's	The cell was exposed to Division Signal and Adrenalin. An NLS is encoded in Exon 2 of the CDKI gene.
T35.	The nucleus glows red.	Matt's Construct	The cell was exposed to Division Signal and Adrenalin. An NLS is encoded in Exon 1 of CDKI.
T36.	The cytoplasm glows red and green.	Matt's Construct	The cell was exposed to only the Division signal. No NLS is present in the CDKI protein.
T37.	The nucleus glows red.	Keisha's Construct	The cell was exposed to Division Signal and Adrenalin. The NLS is encoded in Exon 1.

Problem [38-43] These problems provide a Result and the Experimental Conditions, and your job is to determine which constructs must have been used to produce those results, given the conditions stated.

The answers you can provide for each problem are:

- Only Keisha's construct could have been used.
- Only Matt's construct could have been used.
- Either Keisha or Matt's construct could have been used. The results stated could have been produced with either construct.
- Both constructs must have been used in the cell together.
- No construct or combination of constructs could have produced the results stated.

	<u>Results</u>	<u>Construct(s)</u> <u>Used??</u>	<u>Experimental Conditions</u>
B38.	The nucleus glows red and green.	???	The cell was exposed to only the Division Signal. A NLS is encoded in Exon1 of the CKDI gene.
E39.	The cytoplasm glows red and the nucleus glows red and green.	???	The cell was exposed to only the Division Signal. The NLS is encoded in Exon 3 of the CKDI gene.
E40.	The nucleus glows green and the cytoplasm glows red.	???	The cell was exposed to Division Signal and Adrenalin. The NLS is present in Exon 2.
C41.	The nucleus glows red.	???	The cell was exposed to Division Signal and Adrenalin. An NLS is encoded in exon 3 of CDKI.
E42.	The cytoplasm glows red.	???	The cell was exposed to only the Division signal. No NLS is present in the CDKI protein.
C43.	The nucleus glows red.	???	The cell was exposed to Division Signal and Adrenalin. The NLS is encoded in Exon 3.

First Practice

Introduced in class

Assessed with Clicker Questions



First Practice

Introduced in class

Assessed with Clicker Questions

Second Practice

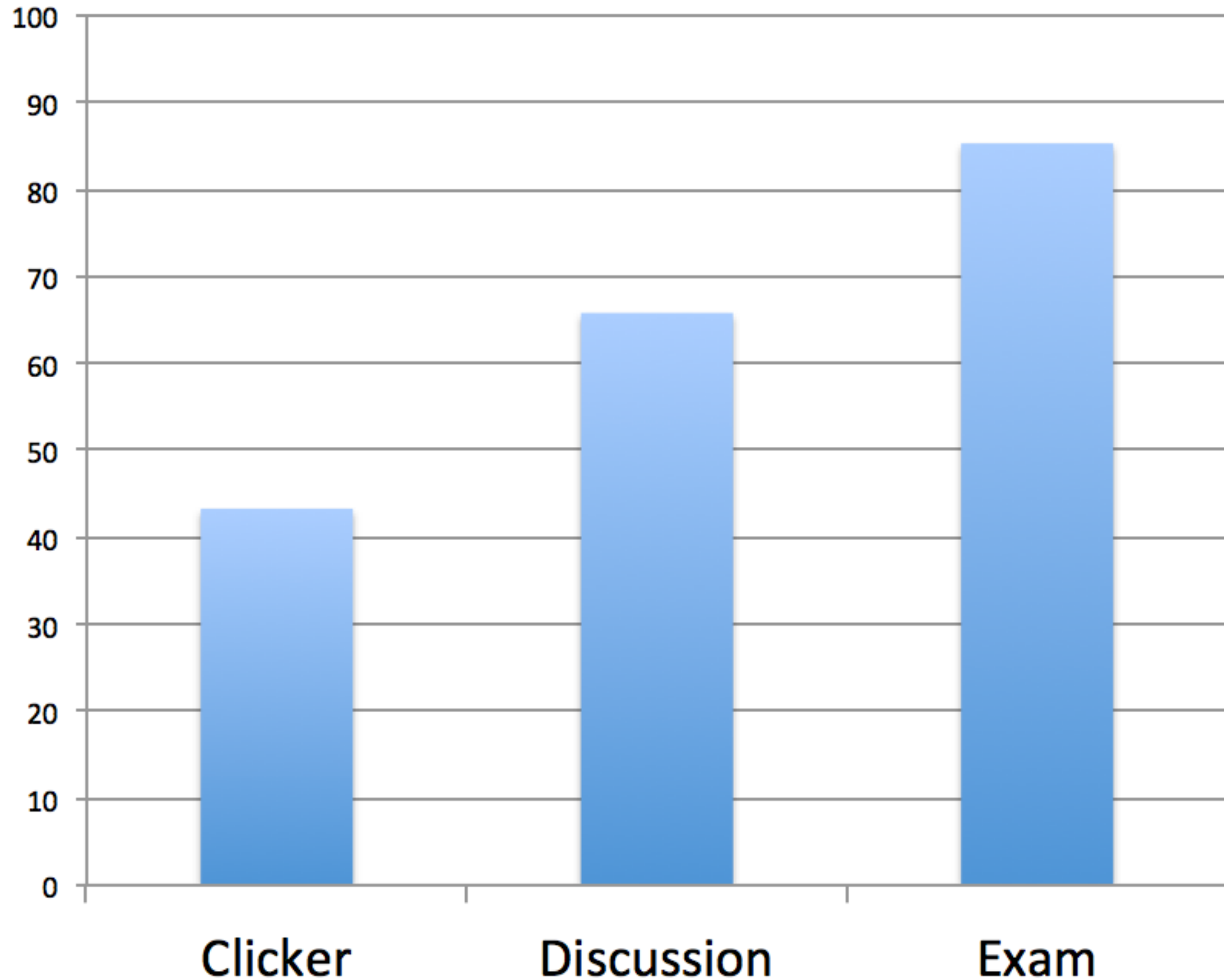
Worksheets in Discussion section

Take Discussion Triple Quiz

Final Assessment

Exam 3

Improvement on Alt Splice Distant Causation Model



Learning Objectives

Engage complex system spanning multiple related models.

Systems thinking - chunking sub-processes into unified concepts

Engage Inductive reasoning to interpret observations in the context of underlying systems.

